Bulgarian Journal of Veterinary Medicine, 2016, **19**, No 1, 19–29
ISSN 1311-1477; DOI: 10.15547/bjvm.927

Original article

GENES EXPRESSION PROFILES IN THE LOIN MUSCLE OF MANYCH MERINO SHEEP WITH DIFFERENT LIVE WEIGHT

V. TRUKHACHEV[1], V. BELYAEV[1], A. KVOCHKO[1], A. KULICHENKO[2], D. KOVALEV[2], S. PISARENKO[2], A. VOLYNKINA[2], M. SELIONOVA[3], M. AYBAZOV[3], S. SHUMAENKO[3], A. OMAROV[3], T. MAMONTOVA[3], N. GOLOVANOVA[1], O. YATSYK[1] & A. KRIVORUCHKO[1]

[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

Summary

Trukhachev, V., V. Belyaev, A. Kvochko, A. Kulichenko, D. Kovalev, S. Pisarenko, A. Volynkina, M. Selionova, M. Aybazov, S. Shumaenko, A. Omarov, T. Mamontova, N. Golovanova, O. Yatsyk & A. Krivoruchko, 2016. Genes expression profiles in the loin muscle of Manych Merino sheep with different live weight. *Bulg. J. Vet. Med.,* **19**, No 1, 19–29.

Genomic breeding methods of sheep require the identification of new genes that affect the quality of meat. Using technology of reverse transcription-quantitative real-time PCR (RT-qPCR) we investigated the expression of 48 genes in the loin muscle. Samples were collected from 19 rams (10 with high and 9 with low live weight) of Manych Merino sheep breed, bred in Russia. The genes GAPDH, PYGM, CAPN3, CAST, ATP5G1, CALM2, SOD1, ASIP and VEGFA showed the highest expression. The group of genes with a medium level of expression included ATOX1, CAPN1, GHR, IGF2, SS18L2, YWHAZ, MYOD1, FOS, CAPN2, GGTA2P, SLC2A3, C-MET, ACVR2A, DGAT1, TLR6, IGF1, ABCG2, FST, BEGAIN. Low levels of expression had genes PYGL, OXTR, BAMBI, PPARG2, SPP2, MSTN, CDKN1A, TGFB1, CYP2J, CXCR4, FGF5, LEPR, IGFBP4, GH, SERT, TSHR. Practical absence of expression was detected for the genes BMP15, FGF7, GDF9 and SST. A significant relationship between the expression level with the live weight of investigated animals was found for FGF5 and GH genes. As a result of the research we suggest investigated genes as new candidate genes, and the study of their structure will permit to use them as genetic markers in developing new breeds of sheep.

Key words: expression, gene, meat, musculus longissimus, PCR, sheep

INTRODUCTION

Nowadays, a number of genes, the structure of which is related to the parameters of meat productivity in sheep are known. Most of them belong to the group of growth regulators of muscle fibres – the genes of myostatin, calpastatin, calpain,

and others. Revealing individual alleles in their structure allows the identification of mutations associated with high meat quality in sheep. However, is not always possible to predict with sufficient accuracy the quality of productive animals only through evaluation of the structure of these genes which indicates the need to search for new candidate genes whose function is associated with the development of the muscle fibres in sheep (Moradi *et al.*, 2012; Miao & Luo, 2013).

The identification of genes that play important role in the growth of muscle tissue of sheep may be during the study of expression profiles by estimating the amount of mRNA of certain genes in muscles. The expression profile reflects the activity of the functioning of individual genes and characterises the synthetic processes in muscles, different in some sheep breeds. There are high chances that the changes in the structure of genes with high expression levels have a more significant impact on the growth and development of muscle tissue in sheep, as shown by Hamill *et al.* (2012) in pigs.

Using Affymetrix Bovine Expression Array technique, Fleming-Waddell *et al.* (2007) have found a particular gene expression profile in skeletal muscle in sheep with callipyge mutation. Studies of genes expression profiles in muscles of sheep are provided by Lobo *et al.* (2012), which revealed different expression of genes MyoD1 and IGFBP4, associated with the breed of sheep and their productive qualities. Zhang *et al.* (2013) conducted a study of gene expression in two sheep breeds by sequencing and revealed significant differences in more than 1,300 genes. Continued research has allowed them to describe 34 genes with different expression related to the development and differentiation of muscle cells (Zhang *et al*., 2014).

In the Russian Federation there are a number of sheep breeds, bred by local breeders and adapted to life in the steppes territory of the North Caucasus. One such breed is the Manych Merino, registered in 1993. Sheep of this breed are widely used in different climatic zones in all categories of farms. Their distinguishing features are the stable breeding qualities and high productivity in the arid steppe zone of the North Caucasus (Babichev & Moroz, 1992; Surov & Aboneev, 2009).

The study of the structure of genes related to meat productivity and evaluation of their expression in the muscle of the Russian sheep breeds was not carried out. In our study we used Dynamic Array Gene Expression technology by Fluidigm (USA) for reverse transcription-quantitative real-time PCR (RT-qPCR) to compare gene transcription profiles in the loin muscle (musculus longissimus) of Manych Merino sheep breed.

MATERIALS AND METHODS

Animals and sample collection

All work was provided in the Genetic Laboratory of Veterinary Care Center (Stavropol State Agrarian University, Russian Federation). We have investigated 19 Manych Merino rams (n=19), one year of age, from a livestock breeding farm of Stavropol Krai, Russian Federation. All animals were healthy, were kept in optimal conditions and were fed a total mix ration. In order to obtain more significant data about variants of genes expression profiles we selected for the research 10 animals with maximum live weight, and 9 animals of the same population with a minimum live weight. After the slaughter, samples from the centre of loin

muscles about 1×1×1 cm were transported to lab.

mRNA collection and cDNA preparation

RNA was isolated from a sample weight of 0.1 g by extraction with TRIzol Reagent («Life Technologies», USA) according to the manufacturer's protocol.

Reverse transcription was performed by using Reverse Transcription Master Mix Kit (Fluidigm, USA). To apply a set of preamplification PreAmp Master Mix and TaqMan Assays (Fluidigm, USA). For amplification was used T100 Thermal Cycler (BioRad, USA).

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

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

Primers to 48 target genes (Table 1) were developed by Fluidigm, USA. To perform real-time PCR, 96.96 Dynamic Array Gene Expression (Fluidigm, USA) were used. Preparation array was performed on Fluidigm IFC Controller (Fluidigm, USA), for real-time PCR and accounting of results used Biomark HD System (Fluidigm, USA) with negative controls in accordance with the equipment manufacturer protocols and reagents. Analysis of gene expression was performed by using Fluidigm Real-Time PCR Analysis Software (Fluidigm, USA). The software provides amplification curves, colour-coded heat maps and cycle threshold (C_t) data for each run. The default cutoff of 0.65 was the arbitrary value for taking C_t into account.

Statistical analysis

For statistical analysis used Student's *t*-test in MS Excel for Windows statistical plug-in. Differences were considered statistically significant if $P<0.05$.

RESULTS

In our study we evaluated the expression of 48 genes involved in the regulation of growth and development of cellular structures by the number of synthesised mRNA in loin muscle tissue of the Manych Merino sheep breed. Results of accounting RT-qPCR, represented by Fluidigm Real-Time PCR Analysis Software in the form of individual expression profiles of studied genes are shown on Fig. 1.

Based on the analysis of digital data of the expression levels of the studied genes, they were divided into three groups: genes with a high level of expression (C_t from 9 to 12), medium level of expression (C_t from 12 to 16) and low level (C_t more than 16) (Tables 2–4).

The highest expression in loin muscle was that of the GAPDH gene. The smallest difference between the minimum and maximum value of C_t among genes with high expression of the examined animals was found in the gene CAPN3, less than 2.5. The greatest difference in C_t of some individuals was observed in genes CAST and ATP5G1, it was nearly 3.8 (Table 2).

Among the genes with medium level of expression (Table 3) the difference in the expression intensity (performance C_t) was the least for genes SS18L2, CAPN2, GGTA2P, ACVR2A and DGAT1. For these genes the difference between the minimum and the maximum C_t value did not exceed 2. The greatest difference in terms of the expression of individual animals was identified in this group for genes IGF2, MYOD1, FOS, SLC2A3

Table 1. List of investigated genes

No	RefSeq ID	Gene name	Full gene name	Chromosome	Position
1.	NM_001078657	ABCG2	ATP-binding cassette, sub-family G (WHITE), member 2	Chromosome 6	36,514,210-36,556,824
2.	NM_001009293	ACVR2A	Activin receptor IIA	Chromosome 2	160,457,581-160,548,685
3.	NM_001134303	ASIP	Agouti-signaling protein precursor	Chromosome 13	63,237,431-63,242,627
4.	NM_001009429	ATOX1	*Ovis aries* ATX1 antioxidant protein 1 homolog (yeast)	Chromosome 5	60,474,740-60,479,122
5.	NM_001009396	ATP5G1	ATP synthase F(0) complex subunit C1, mitochondrial	Chromosome 11	37,173,130-37,175,267
6.	NM_001009761	BAMBI	*Ovis aries* BMP and activin membrane-bound inhibitor homolog (*Xenopus laevis*)	Chromosome 13	35,117,182-35,118,653
7.	NM_001009766	BEGAIN	Brain-enriched guanylate kinase-associated	Chromosome 18	64,087,503-64,094,914
8.	NM_001114767	BMP15	Bone morphogenetic protein 15	Chromosome X	50,970,938-50,977,454
9.	NM_001009759	CALM2	Calmodulin 2 (phosphorylase kinase, delta)	Chromosome 14	52,810,351-52,818,431
10.	NM_001127267	CAPN1	*Ovis aries* calpain 1, (mu/I) large subunit (CAPN1)	Chromosome 21	42,712,976-42,740,799
11.	NM_001112817	CAPN2	Calpain-2 catalytic subunit	Chromosome 12	25,191,495-25,241,603
12.	NM_001009212	CAPN3	*Ovis aries* calpain 3, (p94)	Chromosome 7	34,747,153-34,805,210
13.	NM_001009788	CAST	*Ovis aries* calpastatin	Chromosome 5	93,354,399-93,484,087
14.	NM_001161880	CDKN1A	*Ovis aries* cyclin-dependent kinase inhibitor 1A (p21, Cip1)	Chromosome 20	10,678,753-10,680,684
15.	NM_001111071	C-MET	*Ovis aries* growth factor receptor c-met	Chromosome 4	51,540,365-51,625,496
16.	NM_001277168	CXCR4	C-X-C chemokine receptor type 4	Chromosome 2	173,602,065-173,605,247
17.	NM_001077210	CYP2J	*Ovis aries* cytochrome P450, family 2, subfamily J	Chromosome 1	34,675,227-34,712,820
18.	NM_001110164	DGAT1	Diacylglycerol O-acyltransferase 1	Chromosome 9	13,566,142-13,575,279
19.	NM_001246263	FGF5	Fibroblast growth factor 5	Chromosome 6	94,584,400-94,605,575
20.	NM_001009235	FGF7	*Ovis aries* fibroblast growth factor 7	Chromosome 7	57,779,972-57,841,735
21.	NM_001166182	FOS	FBJ murine osteosarcoma viral oncogene homolog	Human chromosome 14	75,278,774-75,282,230
22.	NM_001257093	FST	Follistatin	Chromosome 16	25,630,860-25,636,124
23.	NM_001190390	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	Chromosome 3	207,818,504-207,822,814
24.	NM_001142888	GDF9	Growth differentiation factor 9	Chromosome 5	41,841,034-41,843,517
25.	NM_001009764	GGTA2P	Glycoprotein, alpha-galactosyltransferase 2, pseudogene	Chromosome 3	14,290,904-14,322,530

Table 1 (cont'd). List of investigated genes

No	RefSeq ID	Gene name	Full gene name	Chromosome	Position
26.	NM_001009315	GH	Growth hormone	Chromosome 11	47,540,169-47,541,799
27.	NM_001009323	GHR	Growth hormone receptor	Chromosome 16	31,832,933-32,000,445
28.	NM_001009774	IGF1	Insulin-like growth factor 1	Chromosome 3	171,268,400-171,327,752
29.	NM_001009311	IGF2	Insulin-like growth factor 2	Chromosome 21	48,655,290-48,680,999
30.	NM_001134302	IGFBP4	Insulin-like growth factor binding protein 4	Chromosome 11	40,250,021-40,260,382
31.	NM_001009763	LEPR	Leptin receptor	Chromosome 1	40,760,256-40,858,312
32.	NM_001009428	MSTN	Myostatin	Chromosome 2	118,144,443-118,149,433
33.	NM_001009390	MYOD1	Myoblast determination protein 1	Chromosome 15	34,370,528-34,371,122
34.	NM_001009752	OXTR	Oxytocin receptor	Chromosome 19	17,656,099-17,664,613
35.	NM_001100921	PPARG2	Peroxisome proliferator-activated receptor gamma	Chromosome 19	56,552,358-56,652,679
36.	NM_001024861	PYGL	Phosphorylase, glycogen, liver	Chromosome 7	40,814,121-40,860,779
37.	NM_001009192	PYGM	Phosphorylase, glycogen, muscle	Chromosome 21	42,295,599-42,307,126
38.	NM_001009446	SERT	*Ovis aries* serotonin transporter	Chromosome 11	20,913,390-20,933,441
39.	NM_001009770	SLC2A3	*Ovis aries* solute carrier family 2	Chromosome 3	206,193,144-206,206,504
40.	NM_001145185	SOD1	*Ovis aries* superoxide dismutase 1, soluble	Chromosome 11	26,456,369-26,456,827
41.	NM_001009224	SPP2	*Ovis aries* secreted phosphoprotein 2	Chromosome 1	6,767,995-6,787,884
42.	NM_001145186	SS18L2	*Ovis aries* synovial sarcoma translocation gene on chromosome 18-like 2	Chromosome 19	14,656,138-14,659,569
43.	NM_001009196	SST	*Ovis aries* somatostatin	Chromosome 1	197,885,693-197,888,971
44.	NM_001009400	TGFB1	*Ovis aries* transforming growth factor, beta 1	Chromosome 14	49,659,752-49,674,730
45.	NM_001135927	TLR6	*Ovis aries* toll-like receptor 6	Chromosome 6	58,034,773-58,037,166
46.	NM_001009410	TSHR	Thyrotropin receptor	Chromosome 7	89,258,424-89,431,877
47.	NM_001025110	VEGFA	*Ovis aries* vascular endothelial growth factor A	Chromosome 20	17,368,866-17,382,112
48.	NM_001267887	YWHAZ	Uncharacterised protein	Chromosome 13	41,293,013-41,293,750

and BEGAIN. The difference in C_t for these genes was more than 5.5, and for gene BEGAIN it was 8.3.

Analysis of the range of individual parameters values of gene expression in loin muscle among a group of genes with low expression levels (Table 4) showed that the difference between minimum and maximum C_t was the lowest for the genes PYGL and PPARG2. In numerical terms, it was less than 2.7. For genes OXTR, BAMBI, SPP2, CXCR4, FGF5 and GH range size of C_t was more than 6.5, while for the GH gene it was the highest of all the studied genes in this group – 7.7.

To assess the connection of expression of individual genes with an integral indicator of productivity of sheep as live weight, animals were divided into two groups – with high and low weight. The average live weight was significantly different between groups at 6.32 kg ($P<0.05$).

The intensity of average expression of each of the two groups of animal genes was classified into three groups: with low expression or non-expressed (Table 4), with an average expression (Table 4) and with high expression (Table 2).

Genes BMP15, FGF7, GDF9 and SST in our study had the lowest, trace amounts of mRNA performance (maximum values C_t or no appearance of luminescence in probes), which allowed considering them practically non transcribed.

Genes LEPR, IGFBP4, GH, SERT and TSHR showed the lowest expression value in which C_t was greater than 19. Three genes – IGFBP4, GH and SERT are located on chromosome 11, the first and second occupied very similar loci.

Relatively low levels of expression were observed in gene MSTN, which is directly related to the regulation of the intensity of the muscle fibres growth.

Analysis of intensity of the individual genes expression related to the live weight showed that the two groups of examined animals were significantly different each from the other only by the intensity of the expression of two genes – FGF5 and GH. The intensity of FGF5 gene expression of

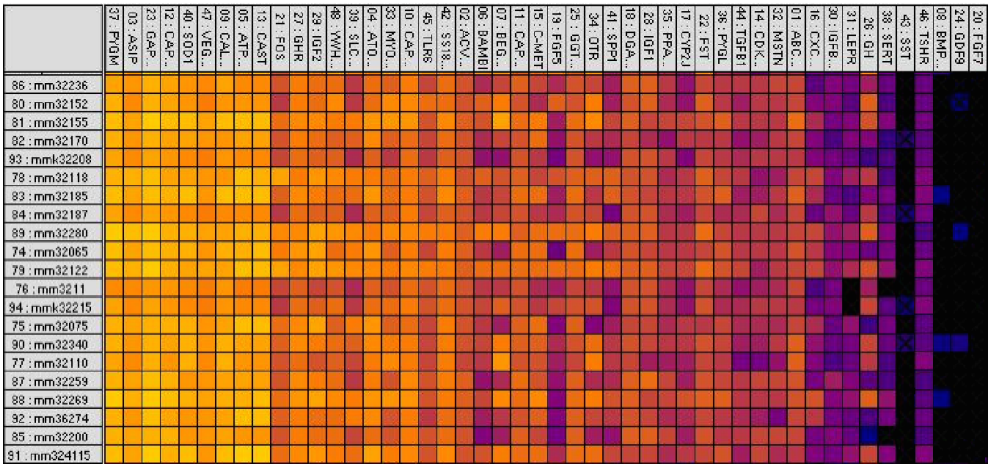

Fig. 1. Individual expression profiles of 19 ram Manych Merino breed. The heat map colours correspond to C_t values: lighter cells indicate higher expression; a black square indicates no C_t value or a value outside of the range. Left column: individual number of animal in farm documentation.

Table 2. High-expression genes in the loin muscle of Manych Merino sheep with high (61.25±0.64 kg) and low (54.93±0.38 kg) live weight. Data are presented as mean±SEM

Gene name	C_t, High weight (n=10)	C_t, Low weight (n=9)	P value
GAPDH	9.42±0.28	9.28±0.30	0.73
PYGM	10.02±0.28	9.75±0.29	0.50
CAPN3	10.33±0.22	10.31±0.25	0.95
CAST	10.33±0.43	10.46±0.49	0.83
ATP5G1	10.35±0.43	10.52±0.49	0.79
CALM2	10.43±0.19	10.58±0.35	0.70
SOD1	10.56±0.28	10.53±0.38	0.95
ASIP	10.88±0.28	11.06±0.36	0.68
VEGFA	11.52±0.27	11.60±0.27	0.82

animals with higher live weight was 14.5% higher than in animals with low weight. The same pattern was found for genes GH, but the magnitude of the differences in this case was 21%.

The expression of other genes with low content of mRNA in muscle was not significantly different between animals with different live weight.

The most intensively expressed genes in the loin muscle of Manych Merino sheep breed were GAPDH, PYGM, CAPN3, CAST and ATP5G1. The expression of these genes in more than 2 times than the level of gene expression TSHR, showed the lowest level of mRNA of transcribed genes in our study.

Table 3. Medium-expression genes in the loin muscle of Manych Merino sheep with high (61.25± 0.64 kg) and low (54.93±0.38 kg) live weight. Data are presented as mean±SEM.

Gene name	C_t, High weight (n=10)	C_t, Low weight (n=9)	P value
ATOX1	12.58±0.32	12.68±0.39	0.85
CAPN1	12.66±0.17	12.64±0.28	0.95
GHR	12.70±0.28	12.63±0.23	0.84
IGF2	12.92±0.61	12.81±0.44	0.88
SS18L2	13.10±0.16	13.08±0.27	0.96
YWHAZ	13.49±0.34	13.04±0.38	0.36
MYOD1	13.91±0.40	13.54±0.55	0.57
FOS	14.29±0.57	14.26±0.76	0.97
CAPN2	14.36±0.20	14.32±0.20	0.89
GGTA2P	14.60±0.28	14.46±0.25	0.69
SLC2A3	14.70±0.67	14.67±0.78	0.97
C-MET	14.78±0.42	14.99±0.56	0.76
ACVR2A	14.82±0.16	14.80±0.13	0.89
DGAT1	14.91±0.23	14.94±0.15	0.92
TLR6	15.26±0.30	14.90±0.43	0.49
IGF1	15.30±0.42	15.08±0.36	0.68
ABCG2	15.36±0.24	15.47±0.29	0.76
FST	15.40±0.26	15.03±0.35	0.39
BEGAIN	15.48±0.85	14.67±0.86	0.49

Table 4. Low-expression genes in the loin muscle of Manych Merino sheep with high (61.25±0.64 kg) and low (54.93±0.38 kg) live weight. Data are presented as mean±SEM

Gene name	C_t, High weight (n=10)	C_t, Low weight (n=9)	P value
PYGL	16.01±0.29	16.09±0.34	0.85
OXTR	16.02±0.76	14.64±0.73	0.18
BAMBI	16.63±0.56	15.78±0.70	0.33
PPARG2	16.79±0.22	16.86±0.24	0.83
SPP2	17.00±0.60	16.77±0.84	0.82
MSTN	17.15±0.46	16.59±0.21	0.26
CDKN1A	17.32±0.45	16.95±0.35	0.50
TGFB1	17.43±0.45	17.20±0.30	0.66
CYP2J	17.44±0.36	17.51±0.49	0.91
CXCR4	18.63±0.69	18.69±0.77	0.95
FGF5	*18.83±0.57*	*16.45±0.77*	*0.02*
LEPR	19.88±0.41	20.22±0.53	0.60
IGFBP4	20.00±0.39	19.90±0.39	0.85
GH	*20.07±1.36*	*16.61±0.81*	*0.04*
SERT	20.61±0.39	21.56±0.57	0.17
TSHR	22.57±0.89	22.98±0.58	0.68
BMP15	Not expressed	Not expressed	
FGF7	Not expressed	Not expressed	
GDF9	Not expressed	Not expressed	
SST	Not expressed	Not expressed	

DISCUSSION

The main task of the study was to evaluate the expression of genes in loin muscle, which is a reference in the definition of a number of parameters that characterise the quality of the sheep meat. Weakly expressed genes may have a different effect on the myocytes. If these are genes of energy metabolism enzymes, it is possible that their lower expression limits the development of muscle fibres. At the same time, for regulatory genes, low expression indices may be enough for a major impact on the size and structure of the muscle (Braun & Gautel, 2011).

We also based our choice of the studied genes on known data for farm animals about their impact on the quality of meat (Kogelman *et al.,* 2011), and the information of the impact on muscle growth in humans and laboratory animals (Braun & Gautel, 2011; Garatachea & Lucía, 2013).

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

Mutations in several of the investigated genes related to the growth and development of muscles in a number of pathological processes in humans and animals, which also points to the possibility of their use as candidate genes for assessing the quality of meat – FOS, YWHAZ, SS18L2.

For gene expression studying there are several methods: sequencing of cDNA, taken by reverse transcription (Wang *et al.,* 2014; Zhang *et al.,* 2014), estimating the number of cDNA by hybridisation on biochips (Lobo *et al.,* 2012) and reverse transcription-quantitative real-time PCR (Sun *et al.,* 2014). Thus, the latter method is the most accurate and is used to validate the results obtained with microarray hybridisation (Lobo *et al.,* 2012). Therefore, to obtain the most accurate results, we used reverse transcription-quantitative real-time PCR on 96.96 Dynamic Array Gene Expression (Fluidigm, USA) in our study.

During the study of gene expression, we have assumed that the maximum intensity of the performance will be at the transcription of genes encoding proteins of energy metabolism and transport systems, and that was confirmed in the analysis of the results. Thus, the maximum expression in this study was found for the gene GAPDH. In their study, Zhu *et al.* (2015) investigated the variability of expression of GAPDH gene in musculus longissimus dorsi of goats. The authors obtained a value of standard deviation of the C_t of about 0.77, which differed little from the value in our research – 0.84.

The PYGM gene was expressed in the loin muscle with the almost same intensity as the GAPDH gene. Two genes, performing regulatory functions of muscle fibres – CAPN3 and CAST had C_t value one unit less than GAPDH as it has been shown in several studies (Azari *et al.*, 2012; Ranjbari *et al.,* 2012). Since they belong to the gene regulatory peptides group, we expected lower rates of expression for them.

In general, in our opinion the group of genes with high expression, despite the absence of differences in the intensity of transcription between animals with different live weight is paramount in relation to the identification of molecular markers of sheep meat productivity. The magnitude of expression of individual value variation may be associated with the presence of allelic variants of genes with different functional activity. Focusing on this figure, we should pay attention in future studies to the structure of the gene ATP5G1, encoding ATP synthase – an enzyme with key position in energy metabolism. The variability of expression of this gene is identical to that of the gene of CAST, whose value for meat sheep productivity is proven.

Among the genes, which have shown in our study average results of the expression activity, the most noteworthy genes are IGF2, MYOD1, FOS, SLC2A3 and BEGAIN. Their high variability of expression may be a consequence of the presence of molecular changes, revealing that we will be able to obtain important markers for genomic selection. As can be seen, MYOD1 was among the genes coding myoblast determination protein 1, directly related to the regulation of myocyte development.

In the group of genes with low expression, in our opinion, the genes OXTR, BAMBI, SPP2, CXCR4, FGF5 and GH deserve a special attention. Also, as in the groups with other expression levels, these genes have shown the maximum range of the individual variability of expression indices. And for the two genes – FGF5

and GH we have found significant differences in expression between the groups with different levels of live weight. The possibility of growth hormone gene expression in muscle has already been proved for a number of animals (Moria & Devlinb, 1999), and the first time we have confirmed it for the sheep.

In investigations of Jeanplong *et al.* (2015) the expression of the MSTN gene in the muscle tissue of sheep was higher than that of IGF1gene. According to our data, the IGF1 gene was expressed with less intensity than MSTN. These differences can be explained by the breed characteristics of animals, or the fact that the authors used semitendinosus muscle tissue for the analysis, whereas we used loin muscle, the activity of the genes transcription of which may be different.

Genes BMP15, FGF7, GDF9 and SST, the expression of which in loin muscle of sheep has not been identified, nevertheless cannot be excluded from the list of gene-candidates affecting the meat quality of sheep. It is possible that they may have a remote action by producing in other tissues. Besides, growth differentiation factor 9 (GDF9) belongs to the same group as the myostatin (MSTN, GDF8), which has a direct and proven impact on the growth and development of muscle tissue.

CONCLUSION

The study of expression of 48 genes in the loin muscle of Manych Merino breed rams has allowed to characterise the animals of this breed in terms of transcription of genes, associated with key regulating processes of muscle growth and performance of enzyme systems of energy metabolism. These data allow to understand the genetic aspects of the muscle fibres of sheep and of mammals in general. The expression of several genes in the muscles has been studied for the first time and the results will be used in further work on the genetics of animals and humans. The main result is the justification of the need for further study of the structure of the investigated genes and identification of their mutations associated with high levels of meat quality of sheep and other farm animals.

ACKNOWLEDGMENTS

This project was funded by Ministry of Agriculture of the Russian Federation (agreement on the procedure and conditions for granting subsidies to financial security, state order for the provision of public services (works) by December 30, 2013 № 3119/13).

REFERENCES

Azari, M. A., E. Dehnavi, S. Yousefi & L. Shahmohamadi, 2012. Polymorphism of calpastatin, calpain and myostatin genes in native Dalagh sheep in Iran. *Slovak Journal of Animal Science*, **45**, 1–6.

Babichev, D. V. & V. A. Moroz, 1992. Wider use of sheep Manychsky type of the Stavropol breed. *Sheep breeding*, **2**, 8–19.

Braun, T. & M. Gautel, 2011. Transcriptional mechanisms regulating skeletal muscle differentiation, growth and homeostasis. *Nature Reviews Molecular Cell Biology*, **12**, 349–361.

Fleming-Waddell, J. N., L. M. Wilson, G. R. Olbricht, T. Vuocolo, K. Byrne, B. A. Craig, R. L. Tellam, N. E. Cockett & C. A. Bidwell, 2007. Analysis of gene expression during the onset of muscle hypertrophy in callipyge lambs. *Animal Genetics*, **38**, 28–36.

Garatachea, N. & A. Lucía, 2013. Genes and the ageing muscle: A review on genetic association studies. *Age*, **35**, 207–233.

Hamill, M. R., J. McBryan, C. McGee, A. M. Mullen, T. Sweeney, T. Talbot, M. T. Cairns & G. C. Davey, 2012. Functional analysis of muscle gene expression profiles associated with tenderness and intramuscular fat content in pork. *Meat Science*, **92**, 440–450.

Jeanplong, F., C. C. Osepchook, S. J. Falconer, H. K. Smith, J. J. Bass, C. D. McMahon & J. M. Oldham, 2015. Undernutrition regulates the expression of a novel splice variant of myostatin and insulin-like growth factor 1 in ovine skeletal muscle. *Domestic Animal Endocrinology*, **52**, 17–24.

Kogelman, L. J. A., K. Byrne, T. Vuocolo, N. S. Watson-Haigh, H. N. Kadarmideen, J. W. Kijas, H. V. Oddy, G. E. Gardner, C. Gondro & R. L. Tellam, 2011. Genetic architecture of gene expression in ovine skeletal muscle. *BMC Genomics*, **12**, 607.

Lobo, A. M. B., S. E. F. Guimaraes, S. R. Paiva, F. F. Cardoso, F. F. Silva, G. A. J. Fernandes & R. N. B. Lobo, 2012. Differentially transcribed genes in skeletal muscle of lambs. *Livestock Science*, **150**, 31–41.

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

Moradi, M. H., A. Nejati-Javaremi, M. Moradi-Shahrbabak, K. G. Dodds & J. C. McEwan, 2012. Genomic scan of selective sweeps in thin and fat tail sheep breeds for identifying of candidate regions associated with fat deposition. *BMC Genetics*, **13**, 10.

Moria, T. & R. H. Devlinb, 1999. Transgene and host growth hormone gene expression in pituitary and nonpituitary tissues of normal and growth hormone transgenic salmon. *Molecular and Cellular Endocrinology*, **149**, 129–139.

Ranjbari, M., A. Hashemi, K. Mardani & R. Darvishzadeh, 2012. Allelic polymorphism of Makoei sheep calpastatin gene identified by polymerase chain reaction and single strand conformation polymorphism. *Journal of Agricultural Science and Technology*, **14**, 533–538.

Sun, W., R. Su, D. Li, H. H. Musa, Y. Kong, J. T. Ding, Y. H. Ma, L. Chen, Y. F. Zhang & W. Z. Wu, 2014. Developmental changes in IGF-I and MyoG gene expression and their association with meat traits in sheep. *Genetics and Molecular Research*, **13**, 2772–2783.

Surov, A. I. & V. V. Aboneev, 2009. On improvement of the Manych Merino sheep breed. *Sheep, Goats, Wool Business*, **3**, 7–9.

Zhang, C., G. Wang, J. Wang, Z. Ji, Z. Liu, X. Pi & C. Chen, 2013. Characterization and comparative analyses of muscle transcriptomes in Dorper and Small-tailed Han sheep using RNA-Seq technique. *PLoS ONE*, **8**, 1–13.

Zhang, C., G. Wang, J. Wang, Z. Ji, F. Dong & T. Chao, 2014. Analysis of differential gene expression and novel transcript units of ovine muscle transcriptomes. *PLoS ONE*, **9**, 1–7.

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

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

Paper received 18.05.2015; accepted for publication 15.06.2015

Correspondence:

Dr. Alexander Krivoruchko
Faculty of Veterinary medicine
Stavropol State Agrarian University
Stavropol, Russian Federation
355017, Zootehnichesky, 12
Phone: +79188814327
Fax: +78652286738
e-mail: rcvm@yandex.ru