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Dlk1 Gene Expression in Different Tissues of Lamb

Research Article

S.H. Masoudzadeh[1], M.R. Mohammadabadi[1*], A. Khezri[1], O.A. Kochuk-Yashchenko[2], D.M. Kucher[2], O.I. Babenko[3], M.V. Bushtruk[3], S.V. Tkachenko[3], R.V. Stavetska[3], N.I. Klopenko[3], V.P. Oleshko[3], M.V. Tkachenko[3] and I.V. Titarenko[3]

[1] Department of Animal Science, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran
[2] Department of Breeding, Animal Genetics and Biotechnology, Zhytomyr National Agroecological University, Zhytomyr, Ukraine
[3] Department of Animal Science, Bila Tserkva National Agrarian University, BilaTserkva, Ukraine

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*Correspondence E-mail: mrm@uk.ac.ir

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ABSTRACT

Delta-like 1 homolog or pre-adipocyte factor 1 (Dlk1) is one of the most significant genes and widely expresses all over mammal's development. Some of the functions identified for *Dlk1*gene are development of muscle, healing of wound, adipocytes proliferation, liver, lung and pancreas development. It also prevents Notch gene conducting toward to govern several operations such like cellular proliferation and differentiation. The aim of this study was to assay the expression of *Dlk1* gene in liver, humeral and femur muscles, brain, adipose, testis and rumen tissues of Kermani lambs. Tissue samples from thirty male lambs of Kermani sheep with approximately the similar weight and age from the Animal Science Research and Training Station of Shahid Bahonar University of Kerman were picked up. Total RNA was isolated, cDNA was synthesized and Real-Time PCR was performed. SAS and REST softwares were used for analyzing the results. The *Dlk1* gene was expressed in all studied tissues of Kermani sheep. The highest expression of *Dlk1* gene expression was observed in liver tissue. There was no statistically significant difference between rumen and femur (leg) muscle, between humeral muscle and liver and between adipose and brain tissue (P>0.05). The lowest expression was related to testicular tissue. Based on results of current study, it can be concluded that this gene has pleiotropic effects with different major and minor outcomes in different tissues. But, for reaching to more decisive conclusion for any tissue, it is necessary to carry out further research noticing various physiological, epigenetic and genetic conditions.

KEY WORDS Dlk1, expression, lamb, real-time PCR, tissues.

INTRODUCTION

Small ruminants, particularly native breed kinds, play a significant role to the livelihoods of a considerable part of human population in the tropics from socio-economic aspects (Ahsani *et al*. 2010; Vajed Ebrahimi *et al*. 2016). Thus, combined trials with emphasis on administration and genetic progress to improve animal outputs are of decisive significance (Zamani *et al*. 2011; Mohammadabadi, 2016). Economical and biological efficiency of sheep production enterprises generally improves by increasing productivity and reproductive performance of ewes (Soufy *et al*. 2009). Twenty-six sheep breeds is bred in Iran (Mohammadabadi *et al*. 2017) including more than 50 million heads (Ahsani *et al*. 2011) that every of which has adapted to particular part of country (Zamani *et al*. 2015). One of the most worth

native sheep breeds is Kermani sheep (Khodabakhshzadeh *et al*. 2016). This sheep has been adapted to stratify and unsuitable circumstances with warm and arid climate, inferior pastures and sparse vegetation level in the southeast of Iran (Ghotbaldini *et al*. 2019). Actual needs of tribesmen and farmers in Kerman province is produced by this dual-purpose medium-sized fat-tailed sheep (meat and wool) with white wool. Thus, attention to breeding of this animal to improve his environmental status and genetic parameters has helped to meet some of the needs of the animals. Along with declined domestic and foreign asks for its wool, the economic worth of this product has decreased in comparison with other products, certainly meat. Thereupon, meat production is currently the capital resource of gain for the sheep breeders (Vajed Ebrahimi *et al*. 2016). Dlk1 (pre-adipocyte factor 1) or Delta-like 1 homolog is one of the most significant genes that widely expresses all over mammals' embryonic development. Based on reports of Bujak *et al*. (2015) and Falix *et al*. (2013), this gene is a transmembrane epidermal growth factor (EGF)-like including an N-terminal signal sequence, six EGF-like repeats, a short juxtamembrane region containing the ADAM17 cleavage site, a short C-terminal cytoplasmic tail (intracellular region) and a transmembrane domain. In pigs, mice, humans and sheep its locus is placed on chromosome 7, 12, 14 and 18, respectively (Oczkowicz *et al*. 2010). Many fetal tissues and undifferentiated cells of murine and humans are place of *Dlk1* gene expression and some of the functions identified for Dlk1gene include development of muscle, healing of wound (Andersen *et al*. 2009), adipocytes proliferation (Smas and Sul, 1993), liver, lung and pancreas development (Tanimizu *et al*. 2003; Yevtodiyenko and Schmidt, 2006) and prevents Notch gene conducting toward to govern several operations such like cellular proliferation and differentiation (Baladrón *et al*. 2005; Nueda *et al*. 2007). *Dlk1*gene is also expressed in the embryonic period and shows negative correlation with the increased level of cellular differentiation and fetal development, when ever does not expressed almost in adult tissues of murine alongside adrenal gland (Smas and Sul, 1993). Pancreas (Jensen *et al*. 1994) and adrenal gland (Jensen *et al*. 1993) are only tissues of adult human in which Dlk1gene is expressed. mRNA of *Dlk1*gene is also expressed in various tissues such as pre-adipocytes (Traustadottir *et al*. 2013), ovary, testis, heart and pituitary gland (Harel *et al*. 2011) and neuron stem cells (Surmacz *et al*. 2012). Among the domestic animals, on the sheep investigation of Dlk1 were extensively carried out and often conducted for finding the single nucleotide polymorphism (SNP) for the callipyge phenotype (CLPG or muscle hypertrophy of the hindquarters). Based on research of Cockett *et al*. (1996) inheritance model of callipyge phenotype is polar over dominance (non-Mendelian) and transfers to offspring only when the father and mother are the origin of mutated allele and wild one, respectively. CLPG mutation multiplies *Dlk1* gene expression level. In livestock, *Dlk1*gene is considered like significant candidate in marker-assisted selection. A sheep with heterozygous genotype in the DLK1-GTL2 domain carrying a paternally inherited mutation has a muscular hypertrophy phenotype which is entirely distinct from the normal phenotype (Amiri Roudbar *et al*. 2018). Practically this phenotype finds only in callipyge flocks, because it is breed specific (Smit *et al*. 2003). On the other hand, Kim *et al*. (2004) showed that Dlk1 polymorphism has correlation with growth, fatness and body composition and inherits as polar-overdominant. Li *et al*. (2008) using quantitative trait loci (QTL) analysis confirmed this association. It has been proven that mRNA expression of Dlk1 in the muscle tissue of broiler is more than its expression in layers, thus it can be concluded that *Dlk1* gene can be considered as new selection-marker for investigation of the high muscle growth in poultry (Shin *et al*. 2009). Since Dlk1expression in farm animals, especially in Kermani sheep is not studied, the aim of this research was to study expression of *Dlk1* gene in different tissues of Kermani sheep.

MATERIALS AND METHODS

In our research, tissue samples containing liver, humeral muscle, brain, adipose, femur (leg) muscle, testis and rumen (3 replications per tissue) from thirty male lambs of Kermani sheep with approximately the similar weight (27.5±0.45 kg) and 6-months-oldfrom the Animal Science Research and Training Station of Shahid Bahonar University of Kerman, Kerman, Iran were picked up. Liquid nitrogen was used for rapidly storing of tissue samples. Extracting total RNA performed applying one Step RNA Reagent Kit (Biobasic Co. Ltd., Iran) based on the guidance of manufacturer. The concentration of extracted RNA was estimated by spectrophotometry at 260 nm, and the absorbance 260 nm:280 nm ratio and electrophoresis on 2% agarose gel stained with ethidium bromide were used for evaluation of RNA quality. RNAs were reverse transcribed with RerertAid™ H Minus First Strand cDNA Synthesis Kit (#K1631, Fermentase Co., Iran) and an oligo d(T) primer was used according to protocol of manufacturer. Amount of total used RNA for the reaction was one microgram. Primers5'-CGTCTTCCTCAACAAGTGCGA-3' and5'-TCCTCCCCGCTGTTGTAGTG-3' for *Dlk1* gene and 5'-GGACATCCGCAAAGACCTGA-3' and 5'-ACATCTGCTGGAAGGTGGACA-3' for beta actin gene was used to perform RT-PCR technique. Samples were amplified using power SYBR green PCR Master Mix. All reactions were performed with optical 96-well skirted mic-

croplates. Reactions were carried out in a volume of 15 µL consisting of 2X SYBR Green PCR Master Mix, 7.5 µL; template cDNA, 1.5 µL; 10 µM forward and reverse primers, 1 µL; ROX, 0.3 µL and ddH2O, 4.7 µL. PCR protocol was done at 94 ˚C for 3 min, then 35 cycles of 94 ˚C for 60 s, 57 ˚C for 60 s, and 72 ˚C for 60 s and final extension at 72 ˚C for 5 min. A standard diagram for *Dlk1* and beta actin genes was drawn for defining quantity of PCR output with distinct concentrations (one, 1/10, 1/100, 1/1000) of cDNA. For *Dlk1* and beta actin genes the PCR reaction yields were 98 and 99%, respectively. For evaluation of Real-Time PCR results, Pfaffl formula, REST (Pfaffl *et al*. 2002) and SAS (2005) softwares were applied.

RESULTS AND DISCUSSION

The extracted total RNA had a good quality and not contaminated, as the 260 nm:280 nm ratio ranged from 1.77 to 1.90. Moreover, all RNA extracted from tissues of the Kermani sheep used in the present study revealed two 18S and 28S bands (Figure 1) and the band intensity of 28S rRNA was almost twice that of other ribosomal bands. This ratio (2:1) is a suitable criterion for indicating good quality of extracted RNA.

The sharp single peaks were observed in the melting and amplification curves of Dlk1 (Figures 2 and 3) and beta actin (Figures 4 and 5) PCR products. Results showed that dimers of primers were not produced and the primers were specific and amplification product was not generated in the negative control sample.

According to the results of this study, PCR amplification curve for *Dlk1* gene samples in different tissues from cycle 22 to 24 began to amplify and enter exponential phase. The PCR products were then introduced into a linear phase, and samples from cycle 30 were then transferred to plateau phase.

The beta-actin gene was used as a housekeeping gene. The PCR amplification curve for the beta-actin gene shows that samples of this gene began to amplify from cycle 12 and entered the exponential phase. In the next step, the PCR products were introduced into the linear phase, and finally samples from this gene were introduced into the plateau phase from cycle 20.

The results of this study showed that samples of this gene produced a peak at 86 ˚C, indicating the production of a specific product in this reaction. The SD and CV values for each tissue are given in Table 1. The fragment size of PCR products for Dlk1 (Figure 6) and beta actin (Figure 7) was 102 bp and 207 bp, respectively.

The *Dlk1* gene was expressed in all brain, humeral muscle, adipose, femur (leg) muscle, rumen and testis tissues of Kermani sheep (Figure 8). The highest expression of *Dlk1* gene expression was observed in liver tissue.

There was no significant difference between the humeral muscle and liver tissues (P>0.05). The lowest expression was related to testicular tissue. There was no statistically significant difference between rumen and femur (leg) muscleand between adipose and brain tissue.

Other investigations have shown that the *Dlk1* gene is expressed in liver (Moore *et al*. 1997; Bauer *et al*. 1998; Kaneta *et al*. 2000; Yevtodiyenko and Schmidt, 2006; Rocha *et al*. 2007; Oczkowicz *et al*. 2010; Falix *et al*. 2013; Charalambous *et al*. 2014), brain (Yin *et al*. 2006; Rocha *et al*. 2007; Oczkowicz *et al*. 2010), adipose tissue (Yevtodiyenko and Schmidt, 2006; Deiuliis *et al*. 2006), muscle tissue (Davis *et al*. 2005; Yevtodiyenko and Schmidt, 2006; Fleming-Waddell *et al*. 2009; Oczkowicz *et al*. 2010; Falix *et al*. 2013; Su *et al*. 2014) and testis (Lottrup *et al*. 2014; Bujak *et al*. 2015; Lottrup *et al*. 2015; Yuan *et al*. 2018) that confirm our results. Yin *et al*. (2006) investigated expression of *Dlk1* gene in the normal brain and in gliomas.

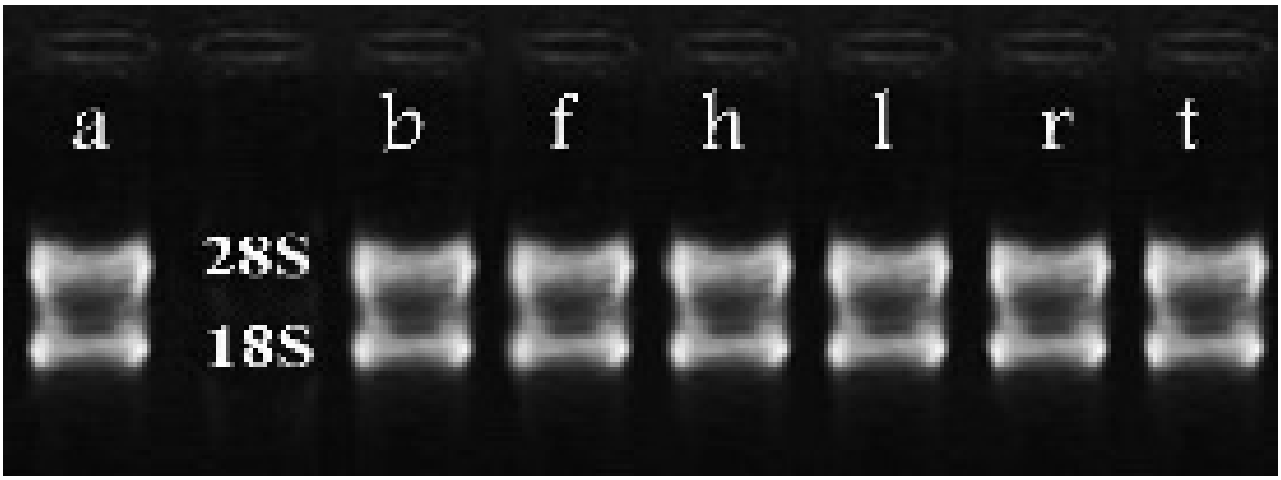

Figure 1 Quality of RNA extracted from seven tissues of Kermani sheep on agarose gel
Samples a, b, f, h, l, r, and t on the figure present adipose, brain, femur (leg) muscle, humeral muscle, liver, rumen and testis tissues, respectively

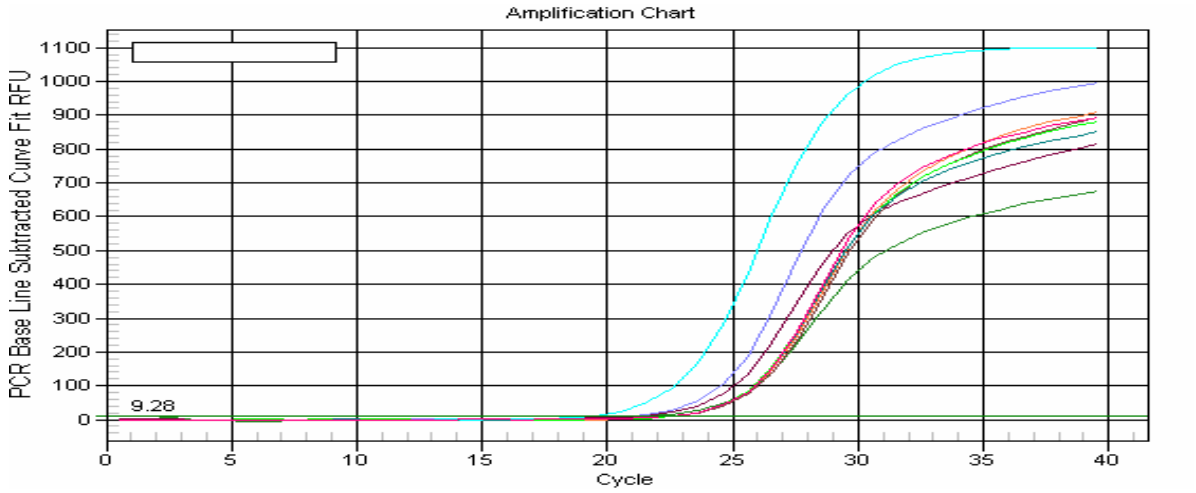

Figure 2 The amplification curve of *Dlk1* gene in the muscle tissue

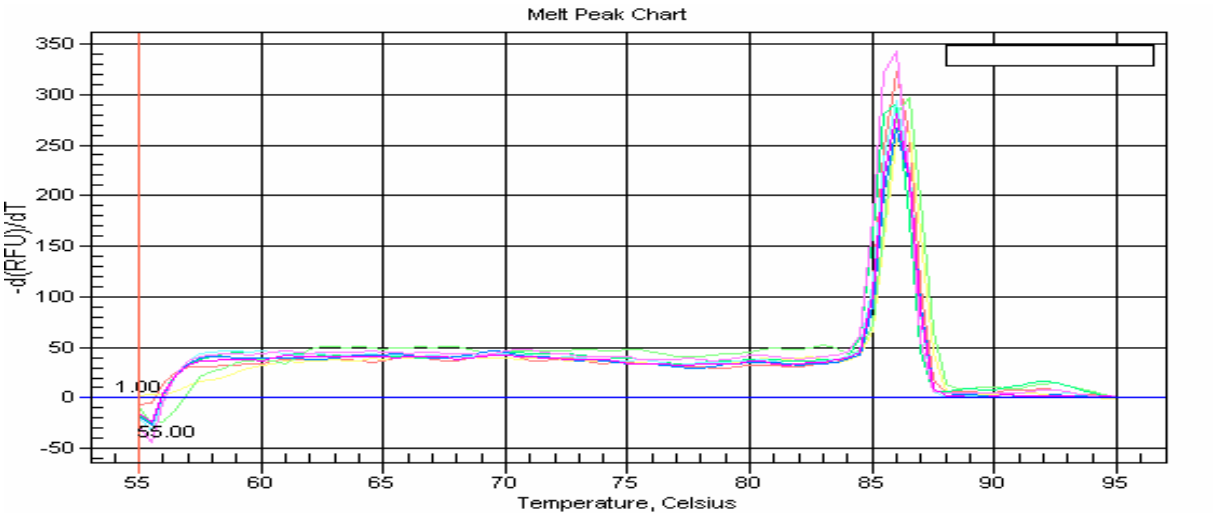

Figure 3 The melting curve of *Dlk1* gene in the muscle tissue

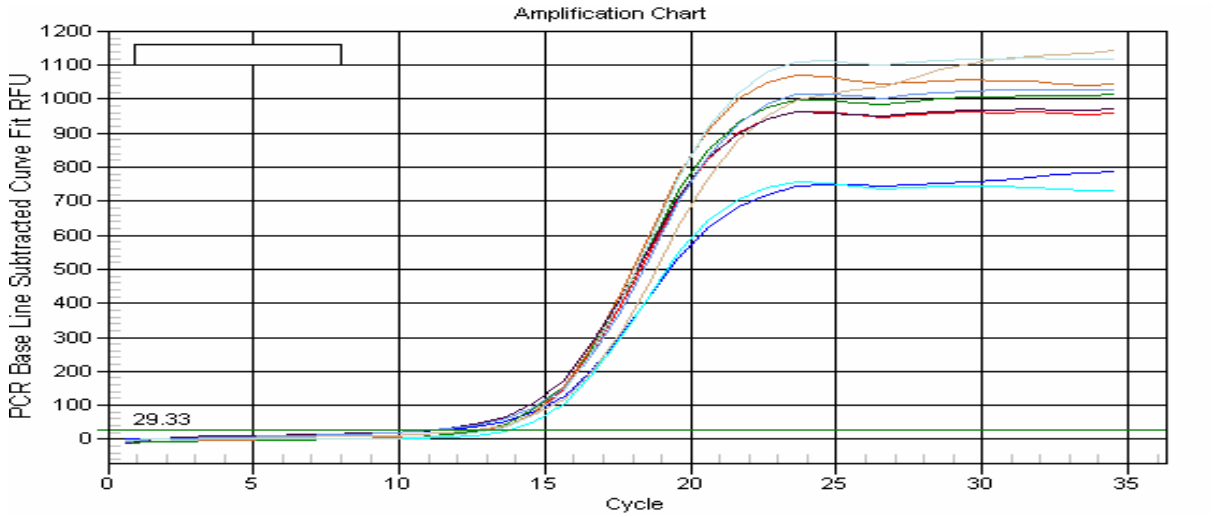

Figure 4 The amplification curve of *beta actin* gene in the muscle tissue

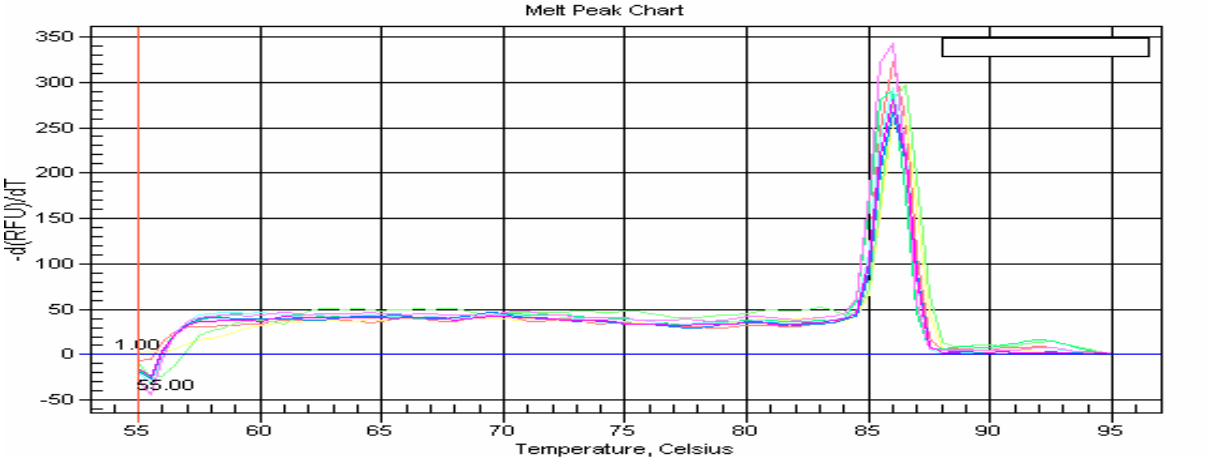

Figure 5 The melting curve of *beta actin* gene in the muscle tissue

In their research *Dlk1* gene expression in gliomas was higher than normal brain (P<0.05). This higher gene expression in glioblastoma multiforme (GBM) was firstly reported by Yin *et al.* (2006) using real time polymerase chain reaction (RT-PCR) and Western blot. They proposed that progression of GBM can be changed by Dlk1. Moreover, it has been illustrated that imprinted Dlk1 is a putative tumor suppressor gene and inactivated by epimutation at the region upstream of GTL2 in human renal cell carcinoma (Kawakami *et al.* 2006).

Although *Dlk1* gene expression in the brain tissue in other studies is in confirmation of our results, but given the conflicting results about the role of this gene in tumor inhibition and its higher expression level in glioma more detailed studies on Dlk1 expression in healthy and cancer tissues are needed. Lottrup *et al.* (2015) reported that Dlk1is expressed in testis with continuous spermatogenesis, where its expression seemed to be increased in samples with leydig cell hyperplasia. Tanimizu *et al.* (2003) showed that *Dlk1* gene has two forms; transmembrane and soluble and this gene plays an important role in the liver development.

Rocha *et al.* (2007) also reported that *Dlk1* gene is expressed in the liver and showed that it could be expressed from the maternal allele at low levels in the liver. In another study, Oczkowicz *et al.* (2010) observed that the pattern of Dlk1 expression in the liver and muscle is the same. All of these reports confirm our results. Overexpression of *Dlk1* gene reduces deficiency in feedback regulation of growth hormone and pituitary insulin-like growth factor 1 (IGF1) persistence (Charalambous *et al.* 2014). This changes increase GH circulatory, which culminates in a switch in whole body fuel metabolism and reduces hepatic steatosis. Hence, *Dlk1* gene mediates important physiological adaptations and metabolic disease resistance.

As demonstrated by Yevtodiyenko and Schmidt (2006) Dlk1 expression in skeletal muscle of sheep during embryogenesis increases but is down-regulated postnatally. These suggested that Dlk1gene plays a significant function in muscle and acts like growth-promoting factor. Davis *et al.* (2005) showed that *Dlk1* gene is overexpressed in skeletal muscle of transgenic mice in comparison with normal mice and lead to significant increase in muscle mass and muscle fiber size.

Table 1 The SD and CV values for *Dlk1* and *beta actin* genes in the studied tissues of Kermani sheep

Tissue	SD		CV	
	Dlk1	Beta actin	Dlk1	Beta actin
Adipose	2.24387467	0.02174956	2.303192	2.409816
Humeral muscle	7.88571267	0.01123356	2.606498	2.264852
Liver	4.60686667	0.00299289	1.939427	1.851060
Brain	2.65284200	1.05684867	2.564759	3.769364
Rumen	1.29961689	1.46104467	2.459380	3.680483
Femur (leg) muscle	7.43057489	0.16234689	3.184865	2.616701
Testis	3.70855800	0.00299289	1.498088	1.851060

SD: standard deviation and CV: coefficient of variation.

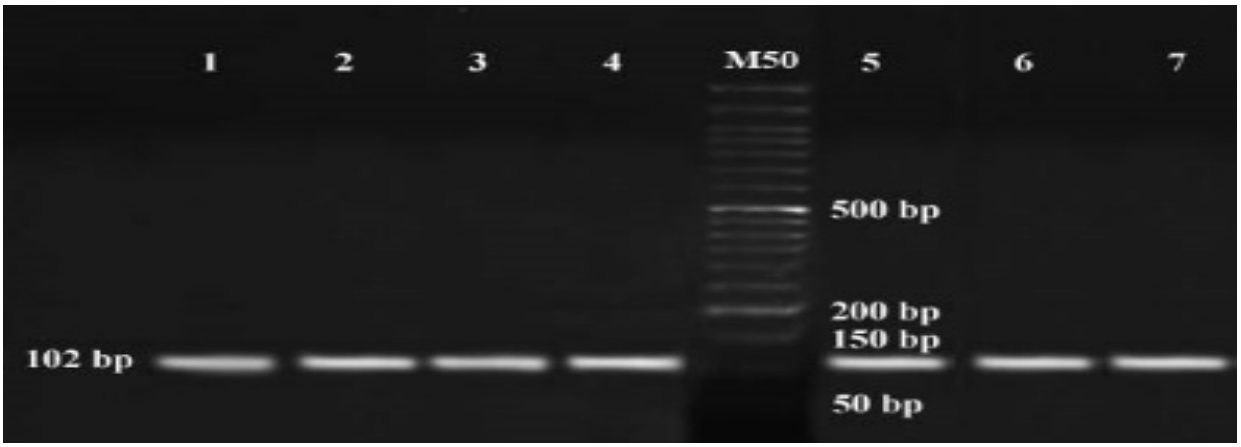

Figure 6 Electrophoresis of studied samples using Dlk1 primers in Kermani sheep on agarose gel
1, 2, 3, 4, 5, 6 and 7 are Dlk1 fragments (102 bp) and M50 is size marker

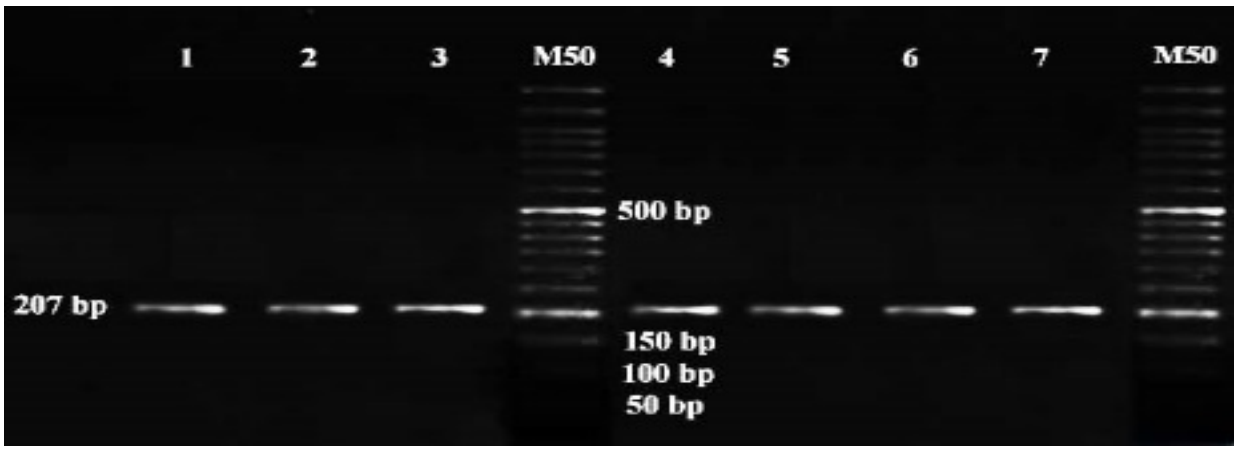

Figure 7 Electrophoresis of studied samples using beta actin primers in Kermani sheep on agarose gel
1, 2, 3, 4, 5, 6 and 7 are beta actin fragments (207 bp) and M50 is size marker

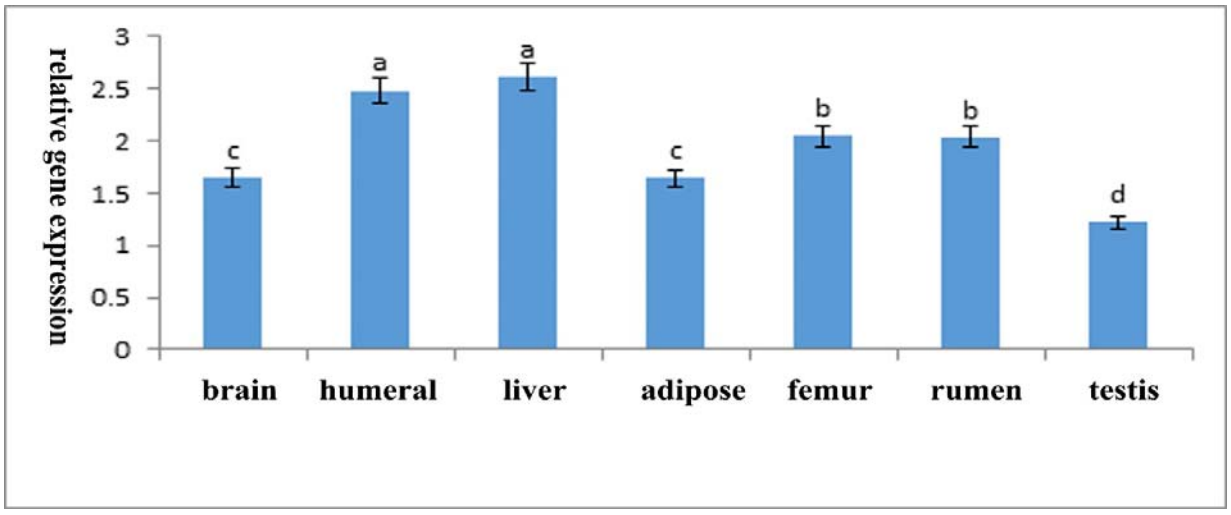

Figure 8 *Dlk1* gene expression in brain, humeral muscle, adipose, femur (leg) muscle, rumen and testis tissues
Comparisons of means (mean of three repeats) were performed using Duncan's test (P<0.05)
The mean of at least one common letter is not statistically significant (P<0.05)

These researchers showed that this gene in callipyge sheep leads to muscle hypertrophy. Su *et al*. (2014) reported that *Dlk1* gene expression positively correlates (P<0.01) with muscle fiber diameter and shear stress, but negatively associates (P<0.01) with muscle fiber density. Muscle fiber diameter had positive and significant (P<0.01) correlation with muscle fiber shear stress, but associated negatively and significant (P<0.01) with muscle fiber density.

CONCLUSION

In general, it can be concluded that the *Dlk1* gene has a differential expression in various tissues. Therefore, complementary experiments and understanding of its mechanisms can be used to improve animal performance using this gene's diversity of expression. Moreover, since *Dlk1* gene plays significant role in various mechanisms, it can be concluded that this gene has pleiotropic effects with different major and minor outcomes in different tissues. But,for reaching to more decisive conclusion for any tissue, it is necessary to carry out further research noticing various physiological, epigenetic and genetic conditions. Also, considering that our study for the first time carried out the Dlk1 gene expression with acceptable and interesting results, therefore the present study opens a new direction for wider investigation in this field.

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