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RESEARCH ARTICLE

microRNA expression patterns associated with average daily weight gain in Kangal Akkaraman Lambs

Gonca Sonmez[1*], Seref Inal[2]

[1]Selcuk University, Veterinary Faculty, Department of Genetics, Konya, Turkey
[2]Selcuk University, Veterinary Faculty, Department of Animal Science, Konya, Turkey

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*goncasen@selcuk.edu.tr

Kangal Akkaraman Kuzularında ortalama günlük ağırlık artışı ile ilişkili microRNA ekspresyon kalıpları

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Öz

Amaç: Bu çalışmada Kangal Akkaraman kuzularında eksprese olan miRNA'ların tanımlanması ve ortalama günlük ağırlık artışı (ADWG) ile ilişkisi olabilecek miRNA'ların belirlenmesi amaçlandı.

Gereç ve Yöntem: ADWG'lerine göre kuzular iki gruba ayrıldı; ADWG'leri 270-412 g (ortalama: 323 g) arasında olanlar yüksek günlük kazanç (HDG) grubu olarak kabul edilirken ADWG'leri 200 g'ın altında olanlar (ortalama: 125 g) düşük günlük kazanç (LDG) grubu olarak kabul edildi. Kuzulardan alınan kanlardan beyaz kan hücreleri ayrıldı ve RNA ekstraksiyonu yapıldı. miRNA kitaplıkları (LDG ve HDG grupları) hazırlandı ve Illumina Novaseq ile dizilendi. Okumalar, Bowtie programı kullanılarak Oar_v3.1 koyun referans genomuna haritalandı. Ayrıca, DESeq R paketi (1.8.3) kullanılarak iki grubun diferansiyel ekspresyon analizi yapıldı. RNA-seq verilerine dayanarak, en yüksek kat artışına sahip iki miRNA seçildi ve ekspresyon seviyeleri kantitatif Real-time PCR (RT-qPCR) ile test edildi.

Bulgular: Toplam 129 bilinen miRNA tanımlandı, 134 yeni miRNA tahmin edildi, 12'si yukarı regüle- 28'i aşağı regüle olmak üzere 40 anlamlı diferansiyel eksprese sahip miRNA tanımlandı (LDG'ye karşı HDG). RNA-seq sonuçlarına göre kat artışı en yüksek iki miRNA tespit edildi; 0,66 (oar-miR-21) ve 0,61 (oar-miR-150). HDG koyunlarının LDG koyunlarına göre 0,63 kat daha düşük oar-mir-150 istatistiksel olarak anlamlı olmayan ekspresyonuna sahip olduğu RT-qPCR ile tespit edildi. Buna rağmen oar-mir-21'in, HDG grubunda istatistiksel olarak anlamlı şekilde 0,40 kat daha az ifade edildiği bulundu ($p < 0,05$).

Öneri: Elde edilen verilerin Kangal Akkraman koyunlarında ADWG ile ilgili ıslah çalışmalarında katkı sağlayabileceği düşünülmektedir.

Anahtar kelimeler: Ortalama günlük ağırşıl artışı, ekspresyon, Kangal Akkaraman, miRNA, RNA-seq.

Abstract

Aim: This study aimed to identify miRNAs expressed in Kangal Akkaraman lambs and to identify miRNAs that may be associated with average daily weight gain (ADWG).

Materials and Methods: According to their ADWG, lambs were separated into two groups: those with ADWGs between 270-412 g (mean:323 g) were considered to have high daily gain (HDG) and those with ADWGs below 200 g (mean:125 g) were considered to have low daily gain (LDG). Blood samples were taken, and white blood cells were processed for RNA extraction. Illumina Novaseq was used to prepare and sequence miRNA libraries (LDG and HDG groups). Readings were mapped. Additionally, using DESeq R package (1.8.3), differential expression analysis of two groups was carried out. Based on the RNA-seq data, the two miRNAs with the highest fold increase were selected and their expression levels were tested by quantitative Real-time PCR (RT-qPCR)

Results: There were identified to be 129 known miRNAs, 134 new miRNAs were predicted, 40 significant differentially expressed miRNAs, of which 12 were up-regulated and 28 down-regulated, were identified (LDG vs HDG). According to RNA-seq results, the two miRNAs with the highest fold change were detected; 0,66 (oar-miR-21) and 0,61 (oar-miR-150). It was determined by RT-qPCR that HDG sheep had 0,63 times lower oar-mir-150 expression, which was not statistically significant, compared to LDG sheep. However, oar-mir-21 was found to be statistically significantly less expressed 0,40 times in the HDG group ($p < 0,05$).

Conclusion: It is thought that the obtained data can contribute to ADWG breeding studies in Kangal Akkaraman sheep.

Keywords: Average daily gain, expression, Kangal Akkaraman, miRNA, RNA-seq.

Introduction

Sheep breeding has been one of the essential branches of animal husbandry for farmers throughout history. It makes significant contributions to the country's economy by producing meat, milk, wool, and leather. The main breeding place of Kangal Akkaraman sheep is the Central Anatolia Region in Turkey. Kangal Akkaraman is a productive sheep breed that produces both meat and milk. Kangal Akkaraman sheep breed, which has long been described as one of the varieties of the Akkaraman breed, is one of the domestic genetic resources of Turkey (Kurar et al 2012). Despite its similarity with the Akkaraman breed, which makes up almost half of Turkey's sheep population, it was accepted and registered as a breed in 2012 due to its morphological head structure, body size and fleece, and physiologically high milk and carcass yield (Kocyigit et al 2018).

MicroRNA (miRNA), which has been detected in almost all animal model systems today, was first observed in *Caenorhabditis elegans* (Lee et al 1993) and is the most common small RNA in living things (Felekkis et al 2010). Single-stranded RNA molecules called miRNAs are brief, non-coding, and only 21–23 nucleotides long. Approximately 30% of all protein-coding genes in mammals are known to be regulated by miRNAs (Filipowicz et al 2008).

Average daily weight gain is associated with muscle development (ADWG). Recent research has demonstrated a substantial correlation between miRNAs and muscle development (Ge et al 2011, Miao et al 2015a, Li et al 2022a). The expression of the genes miR-2387, miR-105, miR-767, miR-432, and miR-433 varies in muscle samples collected at various stages of pregnancy, according to a study on the myogenesis of skeletal muscle in sheep (Yuan et al 2020). Fat deposition also is an important factor associated with ADWG. Studies have been conducted to explain the relationship between fat deposition and miRNA in sheep (Miao et al 2015b, Fei et al 2022). It was found that 54 miRNAs were expressed differentially in a study that looked at miRNAs to understand the variations in fat deposition in two sheep species. In addition, gene ontology and pathway analyzes revealed that Han sheep had less active lipid metabolism compared with the Dorset sheep (Miao et al 2015b). This study was designed to investigate the identification of miRNAs associated to the Kangal Akkaraman sheep breed's ADWG as a local gene source.

Material and Methods

Animals and blood samples

This study was done in 2019 and included thirty-eight male Kangal Akkaraman lambs from Sivas province in Turkey. ADWG was calculated by dividing the live weight difference in a certain period by days [(120th day live weight - birth weight)/120)]. According to their ADWGs, 21 male lambs from the high ADWG (HDG) group and 17 male lambs from the low ADWG (LDG) group were selected for this study. Birth weight (HDG 5,59 ± 1,23 kg; LDG 4,33 ± 0,76 kg), 120th day live weight (HDG 44,3 ± 5 kg; LDG 21 ± 3,4 kg) and ADWG (HDG 323 ± 25 g; LDG 125 ± 25 g) were calculated. At the age of about four months, when the fattening period ended, approximately 10 mL of blood was withdrawn from the jugular vein of the lambs in Ethylenediaminetetraacetic acid (EDTA) tubes.

RNA isolation and small RNA sequencing

Blood samples were processed within 15 min of collection. At 4 °C, samples were centrifuged at 1900 x g for 10 min. The layer containing the white blood cells and platelets that remained between the red blood cells and plasma was collected into 10 mL of cell lysis buffer (140 mM NH4Cl), 10 mM TrisAfter being shaken for 15 minutes at room temperature, it was centrifuged at 1900 x g for 10 minutes at 4 °C. The pellet holding the white blood cells underneath was taken out of the supernatant and placed in liquid nitrogen to be frozen. RNA isolation was performed according to the commercially purchased TRI Reagent (Sigma) protocol. Using a nanodrop device, the RNA samples' quantity and quality were measured, and their suitability was evaluated by visualizing them in an agarose gel. Pools of two groups, LDG (n = 17) and HDG (n = 21), were created from the isolated RNA samples. Once the pooled samples passed the quality control step (Bioanalyzer; RIN, LDG = 8.1, HDG = 7.5), RNA-seq analysis was performed on the Illumina Novaseq platform. RNA-seq results were deposited in the NCBI Gene Expression Omnibus (GEO) database (GSE168662).

Transcriptome assembly and data analysis

For preparing the sRNA library, 3 µg of total RNA per group was used. Using the NEBNext® Multiplex Small RNA Library Prep Set for Illumina®(NEB, USA.), the sequencing library was created. Using DNA High Sensitivity Chips, the Agilent Bioanalyzer 2100 system assessed the quality of the libraries. The TruSeq SR Cluster Kit v3-cBot-HS was used to cluster index-coded samples in accordance with the manufacturer's instructions on the cBot Cluster Generation System (Illumina). After the library had been clustered, the Illumina platform was used to sequence it, producing 50 bp single-ended reads. Perl and Python programs were used to process raw data in the Fastq format. To analyze sRNA reads' expression and distribution over the reference genome (Oar v3.1), Bowtie (Langmead et al 2009) was used to match them to reference sequences. The known miRNAs were looked for using the mapped sRNA tags. miRBase 21 was used as a reference and in order to identify potential miRNA and display secondary structures, customized versions of

the mirdeep2 software (Friedlander et al 2011) and srna-tools-cli were utilized. sRNA markers were matched with RepeatMasker, Rfam database, and sheep breed data to clear repeat sequences, protein-coding genes, tRNA, rRNA, snRNA and snoRNA' sequences from obtained data. The software miREvo (Wen et al 2012) and mirdeep2 (Friedlander et al 2011) were used to predict novel miRNAs.

miRNA expression and differential expression analysis

Transcripts per million reads (TPM) was used to statistically analyze and normalize the expression of known and unique miRNAs in groups (Zhou et al 2010). [Normalized expression = (sample reads * 1 000 000)/total reads].

Functional annotation of the DEGs

DESeq R software (1.8.3) was used to perform differential expression analysis on the two groups (Anders and Huber 2010). The Benjamini and Hochberg (1995) method was used to calculate p-values. The corrected p-value of 0,05 was regarded as statistically significant for differential expression (Storey and Tibshirani 2003). For the Gene Ontology (GO) enrichment analysis, a GO seq-based Wallenius non-central hypergeometric distribution was used, which could correct for the gene length bias (Young et al 2010). The statistical enrichment of the target gene candidates in KEGG pathways was examined using the KOBAS (Mao et al 2005) software.

RT-qPCR analysis

OneScript® Plus cDNA Synthesis Kit (Cat: G270, Canada, ABM) and Poly(A) Polymerase (Cat: E017, Canada, ABM) were used to synthesize miRNA from total RNA. After the Poly(A) Polymerase reaction was completed, 10 µL of the sample was taken and continued with the OneScript® Plus cDNA Synthesis kit. The obtained cDNA was diluted 20-fold and stored at –20 °C for further steps. Forward primers used in the study were designed by replacing uracil with thymine in the mature form miRNA sequences at http://www.mirbase.org/. Commercially available reverse primer (Universal 3' miRNA Reverse Primer, ABM) with forward primers oar-miR-21 (5'-TAGCTTATCAGACTGATGTTGAC-3') and oar-miR-150 (5'-TCTCCCAACCCTTGTACCAGTG-3') were used for RT-qPCR analysis. RNU6 Housekeeping Primers (ABM) were used for normalization. The amplification factors were calculated as 1,98, 1,81 and 1,81 for oar-miR-21, oar-miR-150 and RNU6, respectively (Rebrikov and Trofimov 2006). Reaction buffer contained 10 µL master mix (BrightGreen miRNA qPCR MasterMix-mS, ABM), 300 nM forward/reverse primer and 5 µL cDNA. Sterile ddH2O was added to this mix to raise the final volume to 20 µL. The temperature profile of the reaction was set at 95 °C for 10 min, 40 cycles (10 s at 95 °C, 15 s at 63 °C, 30 s at 72 °C). Melting curve analysis was performed. Each reaction was carried out three times, and the same amount of ddH2O was used instead of cDNA as a negative control. Relative miRNA expression was determined using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittengen 2001). RNU6 was used as the housekeeping, and the control group was evaluated as the LDG group. The significance of the differences between miRNAs was analyzed by independent T-tests (SPSS22). $p < 0,05$ was considered statistically significant.

Results

After sequencing the LDG and HDG RNA-seq libraries, 46,42 million raw reads were collected. The remaining 46,13 million clean reads were used for downstream studies after reads with 5'adapter contamination, low quality reads containing ploy-N, reads without the 3'adapter or the insert tag, and reads with one of ploys A, T, G, or C were removed. Bowtie was used to map clean reads to the Ovis aries reference genome (Oar3.1), where their expression level and distribution on the genome were examined (Figure S1, S2). The percentage of sRNA mapped to the genome was 87,90% and 87,34% for the LDG and HDG groups, respectively. The known miRNA information for each sample was obtained by aligning the mapped reads to the miRBase21. According to data, 129 sRNAs were aligned to miRNA mature sequence (HDG = 125, LDG = 117), 103 sRNAs were aligned to miRNA hairpin sequence (HDG = 103, LDG = 101), and 2923 reads were mapped as unique sRNAs (HDG = 1557, LDG = 1366). Additionally, 134 mature novel miRNAs were also predicted (HDG = 127, LDG = 127). Sequence information of novel miRNAs is given in Table S1.

The read count values were used to analyze the miRNA expression levels between groups and normalized with TPM. oar-miR-21, oar-miR-26a, oar-let-7f, oar-miR-148a, oar-miR-30d, oar-miR-150, and oar-let-7g were found to be highly expressed miRNAs (> 15000 TPM) in sheep white blood cells in the LDG and HDG groups. According to the results of the DESeq R package (1.8.3) analyses, 40 differentially expressed miRNAs were identified (LDG vs. HDG), of which 12 were upregulated, and 28 were downregulated (Table 1). The overall distribution of differentially expressed miRNAs has been given as a Volcano plot (Figure 1).

To identify miRNA expression patterns that might be connected to daily weight gain, cluster analysis was used (Figure 2). According to the heatmap, it was observed that miRNAs exhibiting different expressions had a similar trend of change in the two groups.

Target gene candidates of known and novel miRNAs were predicted using GO enrichment analysis in the ontologies of biological process (BP), cellular component (CC) and molecular function (MF). There were 26 substantially overrepresented GO keywords in the LDG vs HDG comparison (corrected p-value 0.05), including metabolic process (GO:0008152), catalytic activity

Table 1. Differentially expression results of LDG vs. HDG groups

miRNA Name	Log2 Fold Change	p value	Adjusted p value
oar-miR-150	-0,60616	0	0
oar-let-7a	-0,03831	1,60E-135	6,20E-134
oar-miR-25	-0,05752	1,30E-101	3,02E-100
oar-miR-181a	-0,14185	2,34E-90	4,95E-88
oar-miR-27a	-0,06082	1,31E-76	2,54E-75
oar-miR-16b	-0,03972	7,22E-69	1,29E-68
oar-miR-23a	-0,11084	7,91E-65	1,23E-63
oar-miR-30c	-0,07671	1,02E-54	1,48E-53
oar-miR-30b	-0,27132	1,22E-50	1,68E-50
oar-miR-99a	-0,1359	4,78E-43	6,19E-42
oar-miR-106b	-0,17627	4,86E-41	5,97E-40
oar-let-7b	-0,18752	1,80E-35	1,99E-34
oar-miR-23b	-0,39843	4,62E-33	4,89E-32
oar-miR-107	-0,29597	7,50E-29	7,28E-28
oar-miR-29a	-0,22628	7,95E-29	7,41E-28
oar-miR-374b	-0,04205	2,28E-26	2,04E-24
oar-miR-17-5p	-0,05359	1,00E-24	8,65E-24
oar-let-7d	-0,12047	4,26E-15	3,54E-14
oar-miR-152	-0,13719	1,42E-07	1,10E-06
oar-miR-199a-3p	-0,39027	4,39E-03	3,20E-02
oar-miR-194	-0,04943	8,89E-04	6,27E-03
oar-miR-127	-0,37867	3,21E-02	2,20E-01
oar-miR-29b	-0,28874	5,58E-02	3,71E-01
oar-let-7c	-0,13152	2,41E-01	0,000156
oar-miR-379-5p	-0,16478	0,000831	0,005232
oar-miR-411a-5p	-0,05399	0,001646	0,010092
oar-miR-30a-5p	-0,15755	0,001836	0,010969
oar-miR-106a	-0,19286	0,008385	0,048841
oar-miR-21	0,66041	0	0
oar-miR-26a	0,16621	0	0
oar-miR-30d	0,08432	3,64E-226	2,12E-224
oar-let-7f	0,14455	3,04E-208	1,42E-206
oar-miR-191	0,068961	1,18E-110	3,92E-110
oar-let-7g	0,14736	3,73E-108	1,09E-106
oar-miR-103	0,024183	4,40E-103	1,14E-101
oar-let-7i	0,13094	1,50E-66	2,49E-65
oar-miR-22-3p	0,03514	1,25E-38	1,46E-37
oar-miR-26b	0,184	5,42E-31	5,49E-30
oar-miR-221	0,085675	1,38E-09	1,11E-09
oar-miR-374a	0,047508	3,94E-05	2,96E-04

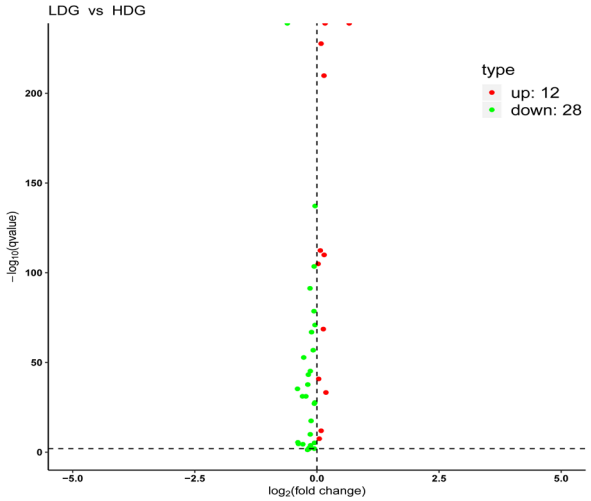

Figure 1. miRNA expression volcano plot between the LDG and HDG groups. The x-axis represents miRNAs in different samples, and the y-axis represents the statistical significance of the difference. Each miRNA is shown by a dot, with blue dots representing no significant difference between miRNAs, red dots representing a significant upregulation of miRNA, and green dots representing a significant difference between miRNAs.

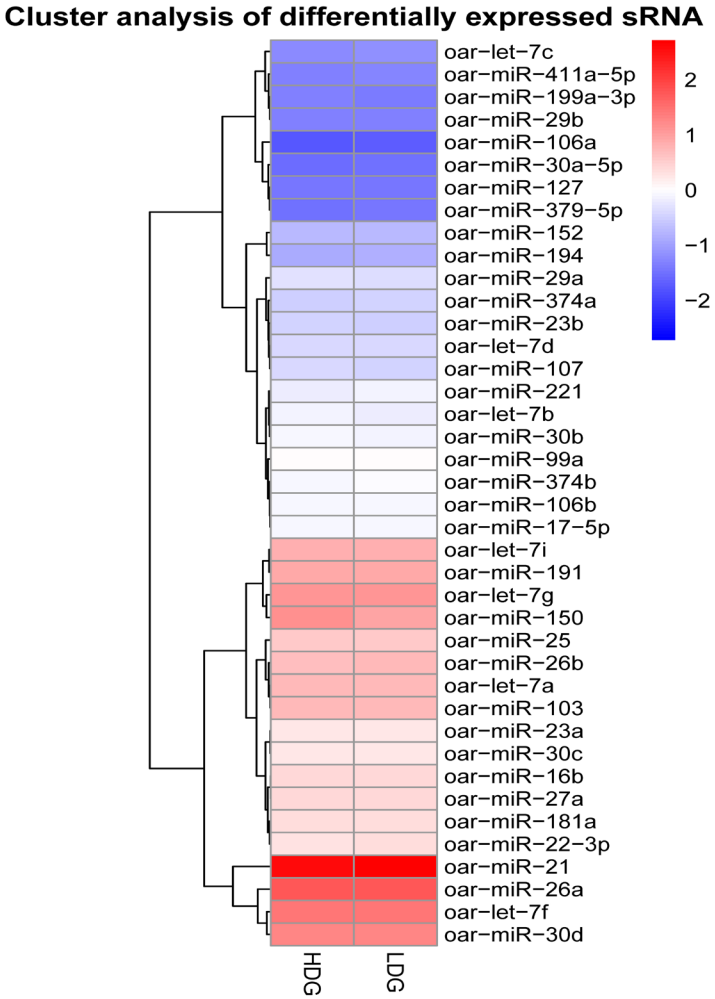

Figure 2. Heatmap of miRNAs that are differently expressed in the LDG and HDG groups. miRNAs with high expression levels are represented by the color red, while those with low expression levels are represented by the color blue. The log10 (TPM+1) value from large to small is represented by the color red to blue.

(GO:0003824), primary metabolic process (GO:0044238), binding (GO:0005488), organic substance metabolic process (GO:0071704), and ion binding (GO:0043167) (Figure 3).

The mechanism of the differential expression of the miRNAs was investigated using KEGG pathway enrichment analysis. Figure 4 presents the outcomes of the KEGG enrichment analysis. The metabolic pathways were associated with the miRNAs that were most significantly up-regulated when the AWDG difference was taken into consideration.

According to the results of RT-qPCR, in which the expression differences of the 2 miRNAs with the highest fold increase were investigated, HDG sheeps had 0,63 times lower oar-mir-150 expression than LDG sheeps, this difference was not statistically significant. Althought, the oar-mir-21 was statistically significantly expressed 0,40 times less in HDG (Figure 5).

Discussion

In 2050, the world's population is projected to reach 9.6

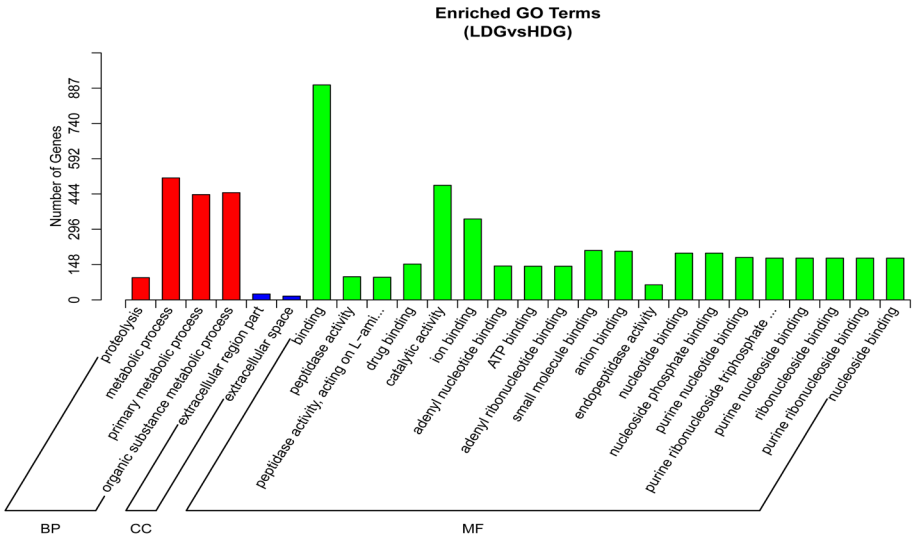

Figure 3. Go enrichment analysis histogram; BP; biological process, CC; cellular component, MF; molecular function

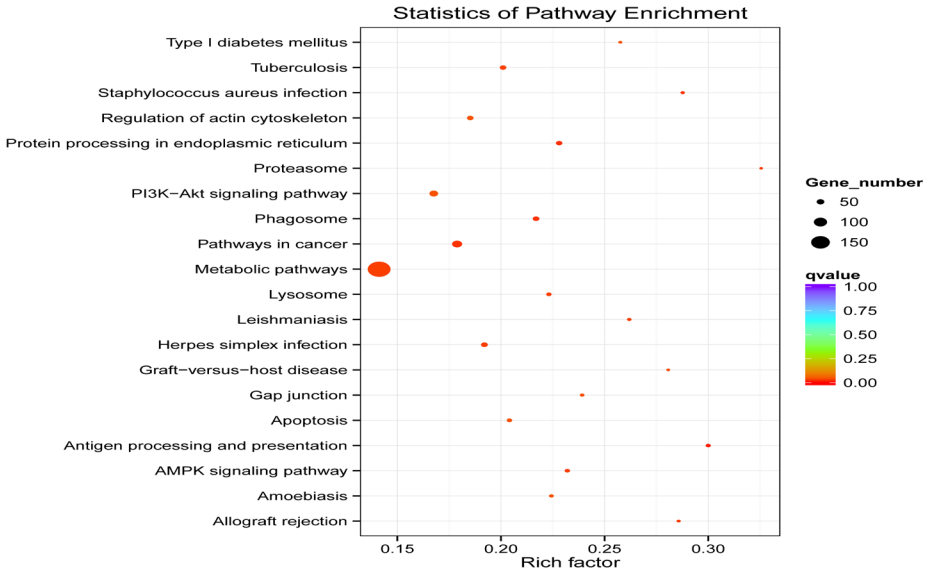

Figure 4. Genes with differential expression shown in a KEGG enrichment scatter plot. LDG vs HDG comparison in KOBAS using enriched KEGG pathways, Fisher's exact test, and Benjamini-Hochberg False Discovery Rate correction. Target gene candidates were considered significantly enriched when the corrected p value was less than 0.05. Rich factor information is displayed on the x-axis, while KEGG pathway information is displayed on the y-axis. The number of DEGs in the pathway is indicated by the size of the point. Different q value ranges are shown by the color of the point.

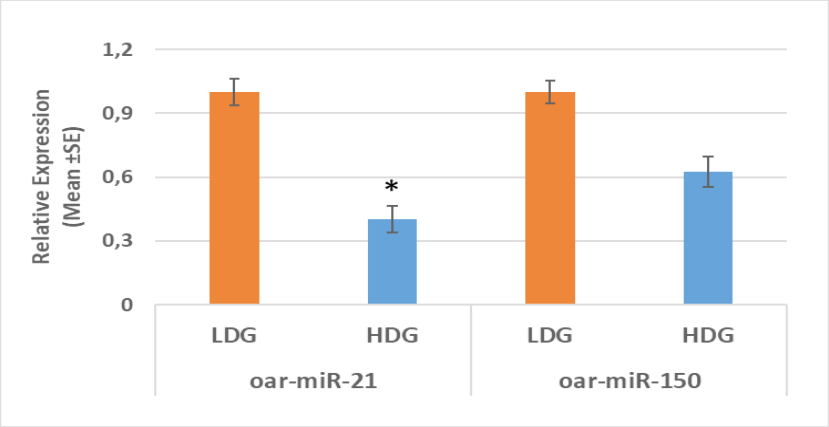

Figure 5. Relative expression differences of oar-miR-21 and oar-miR-150 (SE: Standard error, *: p < 0,05)

billion people and there will be a 58% increase in demand for meat and dairy products (FAO 2011). However, very limited natural resources are available to meet the increasing food demand (Gerber et al 2013). Sheep breeding is important for meat, milk, wool, and skin. It is the preferred animal production area in rural areas because of its low cost (Galal et al 2008). Public Breeding Projects are aimed to determine and improve the yield of the Kangal Akkaraman sheep breed, which differs significantly from the Akkaraman breed in terms of body size and fattening performance. ADWG is one of the parameters used to determine their yield. Although there are numerous published studies on calculating the live weight gains of Kangal Akkaraman lambs (Garip et al 2010), and identifying intra-racial genetic variation at the DNA level (Sahin and Akmaz 2004, Kurar et al 2012), papers citing miRNA studies in Kangal Akkaraman breed are not found in the literature. In this study, miRNAs associated with ADWG were investigated in Kangal Akkaraman lambs, a genetic source of Turkey.

There are many types of RNA with different functions known today, and new RNA molecules are discovered every day. Non-coding small RNA molecules have an important place in this diversity. miRNAs are one of the most important small RNA molecules. These are endogenous, short, non-coding RNAs that link with their target mRNAs to negatively regulate their expression (Bartel 2009). During animal development, hundreds of miRNAs regulate a sizeable part of the transcriptome and a variety of biological processes (Bushati and Cohen 2007, Inui et al 2010).

Sun et al (2019) obtained 11,21 – 15,76 million reads per library while detecting miRNAs associated with growth in muscle tissue of Qianhua Mutton Merino and Small Tail Han sheep. Song et al (2021) studied the expression profiles of miRNAs in the ovaries of six Turpan black sheep during the follicular and luteal phases of the oestrus cycle to investigate the regulatory mechanism of the follicular-luteal phase transition and mapped 86,80% and 62,90% of clean data for each library using the sheep genome (Oar_v3.1). This study obtained 20 958 420 and 25 466 385 raw reads in the LDG and HDG groups of Kangal Akkaraman lambs, respectively. After filtering, the LDG and HDG groups, respectively, obtained 20 788 852 and 25 337 648 clean reads. Further, 18 003 036 and 21 245 522 reads in LDG and HDG groups, respectively, were mapped to the Oar_v3.1 genome. This study's collecting data size was considered suitable for analysis as it was comparable to those of earlier studies. It has been observed that raw and clean reads vary depending on the material used, the platform on which the analysis is performed, and the number of samples used. Sun et al (2019) identified 153 known miRNAs and estimated 8076 novel miRNAs that could not be mapped in the Oar 3.1 genome. Song et al (2021) reported 139 known and 71 new miRNAs in Turpan black sheep. In this study, 129 known miRNAs were detected, and 134 novel miRNAs were predicted in Kangal Akkaraman lambs.

Genetic modifications assist in domesticating livestock and selecting superior breeds (meat quality, yield, disease resistance, etc.). ADWG is known to be parallel to muscle development in terms its efficiency properties. In this study, firstly, miRNAs with different expression in ADWG groups were determined. In LDG and HDG groups, oar-miR-21, oar-miR-26a, oar-let-7f, oar-miR-148a, oar-miR-30d, oar-miR-150 and oar-let-7g were detected as highly expressed miRNAs in sheep white blood cells (> 15000 TPM). It has been discovered that during the development of skeletal muscle, miR-21 targets TGF-1 via the PI3K-Akt-mTOR signalling (Bai et al 2015). Circulating miR-26a has been shown to play a key role in pediatric rhabdomyosarcoma (Tombolan et al 2020). Spear et al (2019), investigating circulating miRNAs associated with abdominal aortic aneurysm, emphasized that let-7f is a potential biomarker. Studies on primary myoblasts from chickens showed that miR-148a-3p could promote differentiation of myoblasts by targeting DYNLL2

and promoting the expression of myosin heavy chain protein (Li et al 2022b). According to several research findings, microRNA-150-5p is closely linked to human skeletal muscle mitochondrial function (Dahlmans et al 2017). The expression of EGR1 in cardiomyocytes is directly regulated by miR-150-5p, according to cell experiments, and EGR1 and miR-150-5p could affect the development of myocardial fibrosis (Shen et al 2019).

Along with muscular growth, ADWG is associated with fat deposition. One study found that miR-21 upregulated HepG2 cells and was physically associated with MEG3 during lipogenesis. MEG3 has been shown to competitively bind with LRP6 to miR-21, then inhibit the mTOR pathway, which induces intracellular lipid accumulation (Huang et al 2019a). miR-26a has been reported to be highly expressed in both subcutaneous and intramuscular fat (Wang et al 2013). According to Huang et al (2019b), let-7f and miR-148a expressions were significantly associated with adipogenesis (Huang et al 2019b). The expression of miR-150 was reduced in mice given a high-fat diet (Zhang et al 2021).

In a previous study, it was found that as mir-21 expression increased, intracellular lipid accumulation decreased (Huang et al 2019a). As a result of this study, the statistical decrease of oar-mir-21 expression in the high ADWG group may be related to intracellular lipid metabolism. According to previous studies, miR-150-5p is associated with skeletal muscle mitochondrial function (Dahlmans et al 2017) and may affect the development of myocardial fibrosis (Shen et al 2019). FGFR1 targeted by miR-150 is important for FGF-mediated proliferation of skeletal muscle satellite cells and contributes to mitogenic effects (Yablonka-Reuveni et al 2015). Although not statistically significant, the expression of oar-mir-150 in high ADWG sheep was decreased, which could indicate that oar-miR-150 is related to the proliferation of muscle tissue.

Regardless of that according to the literature fat deposition and muscle tissue were frequently chosen for growth-related studies, the 40 differently expressed miRNAs found in PBMC in this study imply that blood can potentially be an useful biological material for finding ADWG-related miRNAs.

Conclusion

The data obtained constitute, to our knowledge, the first NGS data related to miRNAs in Kangal Akaraman lambs in the literature. The first stage of growth in sheep, which includes the postpartum and milk sucking stages, is a period in which bone and muscle growth occur. Although the results of miRNA studies that have been carried out using bones and muscles are intriguing, the results of blood studies will be highly usable since blood is readily available material. The results found in this study showed that miRNAs in PBMC are involved in regulating changes in daily weight gain.

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Conflict of Interest

The authors did not report any conflict of interest.

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Author Contributions

Motivation / Concept: Gonca Sönmez, Şeref İnal
Design: Gonca Sönmez, Şeref İnal
Control/Supervision: Gonca Sönmez, Şeref İnal
Data Collection and / or Processing: Gonca Sönmez, Şeref İnal
Analysis and / or Interpretation: Gonca Sönmez
Literature Review: Gonca Sönmez, Şeref İnal
Writing the Article: Gonca Sönmez, Şeref İnal
Critical Review: Gonca Sönmez, Şeref İnal

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