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Bioinformatical Evaluation of PPARA and PPARG Candidate Genes for Milk Quality Characteristics in Turkish Saanen Goats

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Abstract: In this study, genetic information obtained in terms of milk yield and milk quality characteristics was used in order to increase the accuracy of breeding selection of Turkish Saanen goats. For this purpose, DNA sequencing of PPARG and PPARA genes from Turkish Saanen goats were compared with the sequences in gene databases and single nucleotide polymorphisms were examined. A relationship was found between some quality parameters (fat, protein and somatic cell count) of milk measurements of Turkish Saanen goats and PPARA and PPARG gene variants. Single nucleotide polymorphisms were detected in the exons and introns of the relevant gene variants, however, these polymorphisms did not have a statistically significant effect on quality parameters ($p>0.05$).

Key words: Bioinformatics, Candidate genes, PPARA, PPARG, Turkish Saanen goat

Türk Saanen Keçilerinde Süt Kalite Özellikleri ile İlgili PPARA ve PPARG Aday Genlerinin Biyoinformatik Değerlendirmesi

Öz: Bu çalışmada Türk Saanen'i keçilerinin damızlık seçiminde isabet derecesini artırmak amacıyla, süt verimi ve süt kalite özellikleri bakımından elde edilen genetik bilgilerinden yararlanılmıştır. Bu amaçla Türk Saanen keçilerinden alınan kanlardan izole edilen DNA örneklerinden PPARG ve PPARA genlerine ait DNA dizileme yapılarak elde edilen diziler, gen veri bankalarındaki dizilerle karşılaştırılarak tek nükleotid polimorfizmleri incelenmiştir. Türk Saanen keçilerinin süt ölçümlerine ait bazı kalite parametreleri (yağ, protein ve somatik hücre sayısı) ile PPARA ve PPARG gen varyantları arasında ilişki ortaya çıkarılmıştır. İlgili gen varyantlarının ekzon ve intronlarında tekli nükleotid polimorfizmleri tespit edilmiş, bununla beraber bu polimorfizmlerin kalite parametreleri üzerine istatistiksel olarak anlamlı bir etkisi bulunmamıştır ($p>0.05$).

Anahtar Kelimeler: Biyoinformatik, Aday genler, PPARA, PPARG, Türk Saanen keçisi

1. Introduction

The origin of Turkish Saanen goats is based on the Saanen goats brought to Turkey in 1959 for the purpose of improving goat breeding. The Turkish Saanen goat, which was created as a result of crossing Saanen goats and mostly Turkish Hair or Maltese goats in Western Anatolia, is bred as a dominant milk yielding goat in the region. Goat milk contains small fat globules and is easily digestible compared to other milks due to the structural differences of milk proteins. Due to its richness in short and medium chain, single and multi-fatty acids, it is an animal product recommended for infants and the elderly and those with stomach disorders (Haenlein, 2007; Ataç et al., 2018).

In this context, milk yield is one of the most economically important features in Turkish Saanen goat breeding. Along with the amount of milk, components of milk such as protein, fat and the number of somatic cells are among the most important quality criteria affecting the processing of milk (Najafi et al., 2009). Therefore, studies to increase the amount and quality of goat milk are important. There are limited studies conducted with Turkish Saanen goats and generally using morphological and physiological parameters. As a matter of fact, there are many candidate genes associated with growth, reproduction, milk and hair yield parameters in goats (Benjelloun et al., 2015; Işık, 2017; Yakan et al., 2018). Detection of these candidate genes, which can affect milk quality in goat breeding, will increase the accuracy of breeding selection.

Among the candidate genes affecting milk quality, the PPARA (Peroxisome Proliferator Activated Receptor Alpha) gene; it is a gene activated by many fatty acids and fatty acid-derived compounds. Discovered in the early 1990s, hepatic lipid metabolism;

It plays a key role by inhibiting the enzyme 3-hydroxy 3-methyl glutaryl coenzyme A reductase. Synthetic agonists of this gene reduce the plasma triglyceride level and increase the plasma HDL cholesterol level by stimulating the synthesis of apolipoprotein (Apo) AI and Apo-AII in the liver. It is also used in the treatment of dyslipidemia by inhibiting the production of Apo-CIII, a lipoprotein lipase inhibitor (Shipman et al., 2016). Ebrahimi et al., (2015) reported increasing PPARA gene expression in the liver tissue of goats fed diets rich in α-linolenic acid.

The PPARA gene is located on the 5th chromosome in goats and consists of 9 exons. PPARA activation regulates the uptake, utilization and degradation of fatty acids by regulation of genes involved in fatty acid transport, fatty acid binding and activation, and peroxisomal and mitochondrial fatty acid β-oxidation (Kersten 2014). PPARA helps to reduce pain and inflammation by inhibiting the release of various proinflammatory and pro-angiogenic enzymes (D'Agostino et al., 2007). In a study on goats; It was determined that PPARA gene expression increased in the liver tissue of goats fed α-linolenic acid-rich diets (Ebrahimi et al., 2015).

PPARG (peroxisome proliferator activated receptor gamma) is a ligand-dependent transcription factor. They act as sensors of hormones, vitamins, endogenous metabolites and xenobiotic compounds and are involved in the control of expression of many genes as members of the nuclear receptor superfamily. It is known that PPARG plays a role as a transcription factor in fat cell differentiation, fatty acid storage and glucose metabolism (Martin, 2010).

PPARG controls various sequences of biological processes by modulating the expression of specific target genes through a ligand-dependent mechanism (McKenna & O'Malley, 2002). PPARG is involved in the regulation of lipogenic pathways in the mammary gland. (Bionaz et al., 2013). PPARG gene polymorphisms or changes in the expression of this gene can lead to changes in the energy metabolism of the tissues and organs in which the gene is expressed, and may affect milk production and composition. A positive correlation was observed between the increase in milk production during lactation and the stearoyl-coenzyme A desaturase (SCD) enzyme in the mammary glands and PPARG, which regulates its expression in goats (Shi et al., 2013a). In addition, it has been stated that PPARG gene polymorphism affects milk, protein, dry extract and lactose yields and milk lactose content in goats (Ferreira et al., 2020), while in cattle meat tenderness (Fan et al., 2011), meat fatty acid profile and intramuscular It has been found to be associated with fat ratio (Lee et al., 2016).

This gene is involved in the synthesis and secretion of triacylglycerol from mammary gland epithelial cells in goats (Shi et al., 2013a). It has also been reported that PPARG stimulates the synthesis of monounsaturated fatty acids in goat mammary epithelial cells through the control of the enzyme stearoyl-coenzyme A desaturase (Shi et al., 2013b).

The information obtained according to the PPARG and PPARA genes can increase the success of selection studies, as well as save time and cost in breeding studies for the highlighted trait. However, understanding the genetic mechanism in the formation of components such as protein and fat, which also affect the nutritional value of milk, allows changes such as differentiation of nutritional value, reduction or increase of organoleptic properties for consumer demands (Pramod et al., 2018).

In this study, bioinformatics evaluation of PPARA and PPARG candidate genes related to milk yield and milk quality characteristics in Turkish Saanen goats was carried out.

2. Material and Methods

The study was carried out in a private dairy goat farm engaged in intensive breeding in Izmir-Turkey in 2019. The animal material was composed of 40 heads of Turkish Saanen goats, 3 years old and same lactation, with the same environmental conditions in terms of care, feeding and sheltering methods within the enterprise. Blood samples were taken from the vena jugular vein, once, from goats, into tubes with EDTA and without anticoagulant. Milk samples were taken from the milk obtained from the goats that were milked individually in the middle of the periods to represent the first three, 4-5 and 6-9 months of lactation. Goats were followed throughout lactation, milked with a milking machine and milk yields were recorded.

Goats were fed with goat milk feed containing 18 protein 2800 ME (kcal/kg). In the TMR program, a ration was applied according to 17% protein and 2600 ME (kcal/kg) dry matter and milk yield, the goats were fed in the same compartment in the barn and were not taken to the pasture. 1.5 kg of dried alfalfa grass and 1.0 kg of corn silage were used as roughage. According to the daily milk yield of the goats, additional milk feed needs were given individually in the feed vending machine with RFID system.

Dry matter (%), fat (%), true protein (%), total protein (%), lactose (%), non-fat dry matter (%) and

freezing point (°C) in goat milk samples, using Bentley150 device, somatic cell number (cells/mL-milk) was also determined using the Somacount150 instrument (Bentley Instruments, Inc., Chaska, Minnesota, USA). In the Bentley150 device, the composition of the milk is determined by the mid-infrared (MIR) spectrometry technique (Bentley150 Operator's Manual, 1999), in the Somacount150 device the somatic cell count (SHS) is determined by the flow cytometry method (Somacount150 Operator's Manual, 1998) has been detected.

RNA isolation from goat milk somatic cells was performed using "TriPure Isolation Reagent" (Sigma ALDRICH) according to the steps outlined in the instructions for use. Concentrations of isolated RNAs were measured by spectrophotometric methods. cDNA synthesis was performed using Thermo Scientific™ RevertAid RT Reverse Transcription Kit, RNA sample 12 µl for PCR, total 20 microliters, 5X Reaction Buffer 4 µl, RiboLock RNase Inhibitor (20 U/µL) 1 µl, 10 mM dNTP Mix 2 µl , RevertAid RT (200 U/µL) was added to 1 µl tubes.

Primers for PPARA, PPARG and ACTB gene regions taken as reference (control) genes were designed specifically for goat (*Capra hircus*) using the NCBI and ENSEMBLE gene banks. The specificity of the obtained primers was checked with the BLAST (Basic Local Alignment Search Tool, NCBI) program. Primers used for amplification of PPARA, PPARG, ACTB genes are given in Table 1.

Table 1. Primers used for amplification of PPARA, PPARG, ACTB genes
Çizelge 1. *PPARA, PPARG, ACTB genlerinin çoğaltılması için kullanılan primerler*

Gene	Primer	Primer Sequence (5'-3')
PPARA	Forward	GGATCAGATGGCTCCGTTATT
	Reverse	GCAGATCCTACACTCGATGTTC
PPARG	Forward	GGACATTCCGTTCCCAAGAG
	Reverse	GGATACAGGCTCCACTTTGATT
ACTB	Forward	CCCAGCACGATGAAGATCAA
	Reverse	GACAGCGAG GCCAGGAT

The synthesized cDNAs were studied with the Roche LightCycler 480 II instrument using the LightCycler® 480 SYBR Green I Master with primers designed for the indicated gene regions. Real Time PCR components total volume 10 µl Water, PCR-grade 1.9 µl, Forward Primer (F) (intermediate stock 10 µM) 0.3 µl, Reverse Primer(R) (intermediate stock 10 µM) 0.3 µl, Enzyme Mix (LightCycler® 480 SYBR Green I Master) 5 µl, cDNA 2.5 µl were placed in tubes.

In order to detect SNPs in PPARA and PPARG genes, primers used in PCR and Sanger sequence analyzes were designed specifically for Capra hircus using the NCBI and ENSEMBLE gene banks (Table 2). The specificity of the obtained primers was checked with the BLAST program.

Table 2. Primers and DNA sequences used for SNP analysis
Çizelge 2. *SNP analizi için kullanılan primerler ve DNA dizileri*

Primer		Primer Sequence (5'->3')
PPARA	Forward	CGATTCTGAGGCTGTCTAAGG
	Reverse	CGCTGCTGGGTTCTCAA
PPARG	Forward	GCAAAGCGAGTGTTTGTGAAG
	Reverse	TGTGTGGAAAGTGCGGTAAG

DNA samples were studied with PPARA and PPARG specific primers and FastStart High Fidelity PCR System, Thermal Cycler device using dNTPack (Roche) kit. PCR Grade Water 17.25 µl for a total of 25 µl of PCR components, FastStart High Fidelity Reaction Buffer (10x-with 18 mM MgCl2) 2.5 µl, PCR Grade Nucleotide Mix (10mM) 0.5 µl, DMSO 0.5 µl, Forward Primer (intermediate stock) 10 µM) 0.5 µl, Reverse Primer (intermediate stock 10 µM) 0.5 µl, FastStart High Fidelity Enzyme Blend (5U/µl) 0.25 µl, DNA sample was placed in 3 µl tubes. PCR temperature and cycles, initial denaturation 1 cycle, 94°C for 10 minutes, amplification 35 cycles for denaturation 94°C 2 minutes, attachment 57°C 30 seconds and elongation at 72°C 1 minute, final elongation 1 cycle After 7 minutes at 72 °C, it was cooled to 4 °C.

At the end of the PCR analysis, 1.4% agarose gel electrophoresis was performed to see the band formation in the PCR samples. The gel was visualized with a UV Transilluminator after running.

The obtained PCR products were purified using EXOSAP and then sequence PCR was established from the obtained purified PCR products. For an amplification tube (10 µl), the reaction mixture is BigDye Ready Reaction Mix 1 µl, Primer (3.2pmol/µl) 0.5 µl, Distilled water 4.0 µl, 10x Buffer 2 µl, Dye saving 2 µl and Purified PCR product 2.5 µl. has been placed. PCR amplifications were performed for 25 cycles at 96 °C for 10 seconds, at 50 °C for 5 seconds, at 60 °C for 4 minutes and cooled at 4 °C. The resulting sequence PCR products were cleaned with the Zymogen DNA Sequencing Clean-Up kit.

The data obtained at the end of Real Time PCR analysis were analyzed with Absolute Quantification

using Roche LightCycler480 software and normalized values were calculated for target genes for each sample.

Obtained nucleotide sequences were analyzed by multiple sequence alignment method with Molecular Evolutionary Genetics Analysis (Mega X) software and nucleotide polymorphisms were compared with DNA sequences in gene banks with BLAST. Possible single nucleotide polymorphisms (SNP) of gene variants were extracted with bioinformatics programs and their effects were determined.

One Way ANOVA was used in terms of milk components (dry matter, fat, true protein, total protein, lactose, nonfat dry matter, freezing point, somatic cell count, milk yield), target value (T/RPPARA and T/RPPARG) properties; According to the analysis assumptions in question, the normal distributio of the data was tested and transformations were applied for the features that did not show normal distribution.

T/RPPARA for PPARA and T/RPPARG for PPARG and logarithmic transformation for somatic cell number were applied. Since the freezing point properties for dry matter, true protein, total protein, lactose and non-fat dry matter and PPARG did not show normal distribution, SNP differences were analyzed with the nonparametric Mann-Whitney U test. IBM SPSS v25.0 program was used in all statistical analyzes of the study.

3. Results and Discussion

Descriptive statistics for milk components (dry matter, fat, true protein, total protein, lactose, nonfat dry matter, freezing point, somatic cell count), milk yield and target values (T/RPPARA and T/RPPARG) are given in Table 3.

Table 3. Descriptive statistics
Çizelge 3. *Tanımlayıcı istatistikler*

	Mean	Std. Deviation	Minimum	Maximum
T/RPPARA	.00048	.00029	.000000113	.0099
T/RPPARG	.00083	.00050	.000000100	.0165
Dry Matter (%)	11.67	0.25	8.18	16.10
Fat (%)	3.48	0.11	2.18	6.01
True protein (%)	3.49	0.13	2.26	6.79
Total protein (%)	3.72	0.11	2.43	7.37
Lactose (%)	4.67	0.19	3.40	8.86
Non-fat Dry Matter (%)	8.73	0.08	7.92	10.55
Freezing Point (°C)	-.578	0.002	-.550	-.594
Somatic Cell Count (x 1000) cell/mL-milk	899.01	98.62	111	2592
LMY milk (L)	520	77.45	480	650

When evaluated for the PPARA gene, 4 SNPs were determined in the samples examined, and two of these SNPs are located in the gene banks (PPARASNP1-rs640787651 and PPARASNP3-rs665742135). In addition, 2 detected SNPs are not included in the data banks (PPARASNP2 and PPARASNP4). As a result of the SNP analysis performed in the longest exons and intron regions of the PPARA gene, the gene variant registered in the gene banks with the number rs640787651 in the 6th and 7th introns of the PPARA gene was detected in 1 sample. In this gene variant, a single nucleotide polymorphism was seen in which Thymine was replaced by Cytosine. PPARASNP2 was detected in 6th and 7th introns in 1 sample, and there was a change in which Adenine nucleotide replaced Guanine. One SNP registered in the databases with the number rs665742135 has been detected (PPARASN3). It was found that Cytosine nucleotide replaced Thymine nucleotide in this SNP, which was detected in 8 samples and located in the seventh exon, but it was observed that the coded Serine amino acid did not change. In addition, a single nucleotide polymorphism was detected in the 7th and 8th introns of 2 samples, in which Adenine nucleotide replaced the Guanine nucleotide. This gene variant is also not included in the gene banks data (PPARSNP4).

In the analysis for the PPARG gene, 4 SNPs that were not registered in gene banks were found (PPARGSNP1, PPARGSNP2, PPARGSNP3, PPARGSNP4). While two of these SNPs are found in exons, two of them are determined in introns. The most common SNP in the samples for PPARA and PPARG genes is PPARGSNP1 found in 9 samples, and it is located in exon 6. In this single nucleotide polymorphism, it was determined that the Cytosine nucleotide was replaced by the Thymine nucleotide. However, it was determined that the coded Tyrosine amino acid did not change.

PPARGSNP2 and PPARGSNP3 were the only two SNPs detected in the same sample. These polymorphisms occurred in the 6th exon and 6th and 7th introns of the gene and are not available in data banks. In PPARGSNP2 detected in the sixth exon, it was determined that the Thymine nucleotide replaced the Cytosine nucleotide, but there was no change in the coded Phenylalanine amino acid. In the SNP detected in the introns, it was found that the Thymine nucleotide replaced the Adenine nucleotide. PPARGSNP4 was detected in 2 samples and was found in the 6th and 7th introns.

The following statistical models and terms were used for the one-way analysis of variance applied to reveal the SNP differences in the study.

$Y_{ijk}=\mu+a_i+e_{ij}$ (1)

In the equation, μ: the population mean in terms of the tested trait, a_i: i. define the group (SNP) effect and the e_{ij} random error effect.

Variance analysis results are given in Table 4 and Table 5. While the effects of SNP2 on the target value of T/RPPARA and SNP3 on T/RPPARG were significant ($p<0.05$), there was no significant difference between gene expression levels in terms of other characteristics ($p>0.05$).

According to the results of the analysis, the effect of PPARASNP2 was found to be significant in the change of the T/RPPARA ratio used in the analysis of the change in gene expression. Similarly, the effect of PPARGSNP3 change on T/RPPARG change was found to be significant.

The results of the nonparametric Mann-Whitney U test obtained in the study are given in Tables 6 and 7.

Table 4. Significancy levels for PPARA
Çizelge 4. *PPARA için önemlilik düzeyleri*

	PPARASNP1-rs665742135	**PPARASNP2**	**PPARASNP3-rs665742135**	**PPARASNP4**
T/RPPARA	0.755	0.006*	0.523	0.404
Fat (%)	0.188	0.069	0.068	0.109
Somatic Cell Count (x 1000) cell/mL- milk	0.179	0.454	0.792	0.848
Milk (gr)	0.877	0.305	0.100	0.517

*: P<0.05

Table 5. Significancy levels for PPARG
Çizelge 5. *PPARG için önemlilik düzeyleri*

	PPARGSNP1	**PPARGSNP2**	**PPARGSNP3**	**PPARGSNP4**
T/RPPARG	0.617	0.491	0.020*	0.491
Fat (%)	0.996	0.416	0.088	0.416
Somatic Cell Count (x 1000) cell/mL- milk	0.224	0.377	0.550	0.377
Milk (gr)	0.244	0.170	0.451	0.170

*: P<0.05

Table 6. Nonparametric statistics for PPARA
Çizelge 6. *PPARA için parametrik olmayan analiz sonuçları*

	PPARASNP1-rs665742135	**PPARASNP2**	**PPARASNP3-rs665742135**	**PPARSNP4**
Dry Matter (%)	0.567	0.118	0.143	0.286
True protein (%)	0.899	0.014*	0.026*	0.110
Total protein (%)	0.390	0.037*	0.339	0.328
Lactose (%)	0.567	0.044*	0.026*	0.091
Non-fat Dry Matter (%)	0.086	0.144	0.011*	0.374

*: P<0.05

Table7. Nonparametric statistics for PPARG
Çizelge 7. *PPARG için parametrik olmayan analiz sonuçları*

	PPARGSNP1	**PPARGSNP2**	**PPARGSNP3**	**PPARGSNP4**
Dry Matter (%)	0.972	0.155	0.075	0.155
True protein (%)	0.297	0.286	0.445	0.286
Total protein (%)	0.781	0.477	0.126	0.477
Lactose (%)	0.626	0.183	0.702	0.183
Non-fat Dry Matter (%)	0.972	0.534	0.098	0.534

In accordance with the nonparametric analysis results, the effect of PPARASNP2 on true protein, total protein and lactose was significant ($p<0.05$) for PPARA, while the effect of PPARASNP3-rs665742135 on true protein, lactose and dry matter was significant. There was no significant difference between gene expression levels and characteristics for PPARG ($p>0.05$).

On the other hand, target gene and reference gene expression ratio was used to examine the change of gene expression (T/RPPARA and T/RPPARG). Relationships between T/RPPARA, T/RPPARG, and traits were examined using the Pearson correlation test. As a result of the Pearson correlation test of T/RPPARA and T/RPPARG and milk yield values, a significant correlation of 0.05 was found between T/RPPARA and

True protein (%) and lactose (%). Although there was no significant correlation between T/RPPARG and milk yield values, it was determined that there was a negative correlation between dry matter (%), total protein (%) and somatic cell count (x1000 cells/mL- milk).

Relationships between T/RPPARA and rs665742135 gene variant and between T/RPPARG and SNP detected in 6th and 7th introns were investigated by regression analysis. The multiple regression model used in the study is given below:

$$Y= b_0+b_1X_1+ b_2X_2+b_3X_3+ b_4X_4+e_i \quad (2)$$

Where: Y= dependent (T/RPPARA and T/RPPARG) variable, X_i= (i = 1,2,3,4) independent (SNP) variables, b_i= (i: 1,2,3,4) denotes regression parameters and e_i: random error.

While the SNP numbered rs665742135 on T/RPPARA, which is the target value of PPARA, was found to be statistically significant ($p<0.05$), no significant contribution was found for other SNPs ($p>0.05$). While SNP3 was found to be statistically significant ($p<0.05$) on T/RPPARG, the target value of PPARG, other SNPs did not have a significant contribution ($p>0.05$).

Abousoliman et al. (2021) investigated the relationship between genome wide SNP and milk performance characteristics in Egyptian Barki Sheep and stated that PPARA has a critical role in the regulation of milk fat synthesis. While the effect of PPARASNP2 on protein and lactose was significant ($p<0.05$), the effect of PPARASNP3-rs665742135 on true protein, lactose and lean dry matter was significant, so further investigation of these SNPs as candidate genes would be beneficial.

The Peroxisome Proliferator-activated Receptor Gamma gene has a significant effect on the transcription of genes involved in lipid metabolism. Since the genes affected by the receptor are also highly expressed in the mammary glands of organisms, there may be a relationship between them and the characteristics related to milk production (Pramod et al., 2018). According to Shi et al. (2013b) found a significant relationship between the Peroxisome Proliferator-activated Receptor Gamma gene and the regulation of triacylglycerol synthesis in mammary cells in their study in goats. Ferreira et al. (2020) investigated the effects of polymorphisms in PPARG and UCP2 genes on milk yield and composition in goats; They reported that the PPARG-A allele they detected in the PPARG gene positively affected the total milk, fat, protein, dry matter, and lactose content even in heterozygous condition.

Yakan et al. (2018) examined the effects of some candidate genes on mastitis resistance, milk yield, and milk quality in goats, and reported that the expression of the Peroxisome Proliferator-activated Receptor Gamma gene was downregulated in the later stages of lactation. Although there was no significant correlation between T/RPPARG and milk yield values in our study, it was determined that there was a negative correlation between dry matter (%), total protein (%) and somatic cell count (x1000 cells/mL- milk), due to the downregulation of the receptor gene. At the same time, the significant effect of PPARGSNP3 on T/RPPARG change but not significant effect on milk characteristics may be related to sampling period in lactation and downregulation of the gene.

4. Conclusion

In the study, single nucleotide polymorphisms were detected in the exons and introns of the PPARA and PPARG genes, however, it was found that these polymorphisms did not cause any amino acid changes.

According to the results of nonparametric analysis, the effect of PPARASNP2 on true protein was found to be significant ($p<0.05$) for PPARA, but not for other features. There was no significant difference between gene expression levels and characteristics for PPARG ($p>0.05$).

As a result of analysis of variance, the effects of PPARASNP2 on target value T/RPPARA and PPARGSNP3 on T/RPPARG were found to be significant ($p<0.05$), but there was no significant difference between gene expression levels in terms of other characteristics ($p>0.05$). It was determined that the detected SNPs did not change the amino acid. Although the SNP2 effect was found to be significant on the traits examined in this study, it is thought to have no effect on the investigated traits since it was not found to be significant on other traits.

The multiple regression analysis examining the effect of SNPs on the target value of PPARA, T/RPPARA, while the SNP numbered rs665742135 was found to be statistically significant ($p<0.05$), no significant contribution of other SNPs was determined ($p>0.05$).

The Pearson correlation test results of T/RPPARA and T/RPPARG and milk yield values, a significant correlation of 0.05 was found between T/RPPARA and True protein (%) and lactose (%). Although no significant correlation was found between T/RPPARG and milk yield values, a negative correlation was found between dry matter (%), total protein (%) and somatic

cell count (x1000 cells/mL- milk). A positive correlation was found between PPARA gene expression and true protein and lactose values. Since a negative correlation was found between PPARG expressions and dry matter, total protein and somatic cell count, the effect of SNP rs665742135 on PPARA gene expression and PPARGSNP2 on PPARG gene expression. Since the effect on milk yield was found to be statistically significant ($p<0.05$), the relationship between the number of samples and milk yield parameters should be investigated in terms of these genes. In studies examining polymorphisms and gene expressions in PPARG Peroxisome proliferator receptor genes, there are significant differences in terms of goat breed, care and feeding styles of animals, and number of samples (Bionaz et al., 2013).

Introns can also affect mRNA metabolism, initiating transcription of the gene, editing and polyadenylation of the pre-mRNA, translation, and degradation of the mRNA product (Hou et al., 2013). In our study, it was found that PPARGSNP3's single nucleotide polymorphism in introns is important on T/RPPARG value. Although it occurs in introns, it may be useful to examine this SNP with more sample numbers, since its relationship with gene expression is important.

More detailed study of polymorphism in PPARA and PPARG genes and their expression levels in lactation by increasing the number of samples is necessary to identify candidate genes in terms of milk yield and milk yield characteristics.

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References

Abousoliman, I., Reyer, H., Oster, M., Murani, E., Mohamed I., & Wimmers, K. (2021). Genome-Wide SNP Analysis for Milk Performance Traits in Indigenous Sheep: A Case Study in the Egyptian Barki Sheep. *Animals*, 11(6):1671. https://doi.org/10.3390/ani11061671.

Ataç, F.E., Takma, Ç., Gevrekçi, Y., Yeğenoğlu, E.D., & Örgel, S. (2018). Hair Goat Breeding in Ecological Environment and Products: A Review. *International Echology 2018 Symposium*, P:1058, 19-23 June 2018, Kastamonu Turkey. www.ecology2018.com

Benjelloun, B., Alberto, F. J., Streeter, I., Boyer, F., Coissac, E., Stucki, S., Mohammed B., Mustapha, I., Mouad, C., Abdelmajid, B., Kevin, B., Adriana, A., Stefan, E., Abdelkader, C., Laura, C., Paul, F., Stephane, J., Pierre, T., Farnçois, P., & Nextgen, C. (2015). Characterizing neutral genomic diversity and selection signatures in indigenous populations of Moroccan goats (Capra hircus) using WGS data. *Frontiers in Genetics*, 6:107. doi: 10.3389/fgene.2015.00107.

Bentley150 Operator's Manual. (1999). *A guide for installation, operation, and maintenance*. Bentley Instruments, Inc. Chaska, Minnesota, USA.

Bionaz, M., Hausman, G.J., & Mandard, S. (2013). Physiological and nutritional roles of PPAR across species. *PPAR Research*, 1-3.

D'Agostino, G., LaRana, G., Russo, R., Sasso, O., Iacono, A., & Esposito, E. (2007). Acute intracerebroventricular administration of palmitoylethanolamide, an endogenous peroxisome proliferator-activated receptor-alpha agonist, modulates carrageenan- induced paw edema in mice. *Journal of Pharmacology and Experimental Therapeutics*, 322:1137-43.

Ebrahimi-Mamaeghani, M., Mohammadi, S., Arefhosseini, S. R., Fallah, P., & Bazi, Z. (2015). Adiponectin as a potential biomarker of vascular disease. *Vascular Health and Risk Management*, 11: 55-70.

Fan, Y. Y., Sen Zan, l., Fu, C. Z., Tian, W.Q., Wang, H. B., Liu, Y. Y., & Xin, Y. P. (2011). Three novel SNPs in the coding region of PPARγ gene and their associations with meat quality traits in cattle. Molecular Biology Reports, 38 (2011), pp. 131-137.

Ferreira, L., Soaresa, M.A.M., Rodrigues, M.T., de Araujoc, J.L.S., Melod, A.L.P., Gasparinoe, E., & Garciaa, O.S.R. (2020). UCP2 and PPARG gene polymorphisms and their association with milk yield and composition traits in goats. *Small Ruminant Research Volume*, 192, November 2020, 106210.

Haenlein, G.F.W. (2007). About the evolution of goat and sheep milk production. *Small Ruminant Research*, 68: 3-6.

Hou, J.X., An, X.P., Song, Y.X., Wang, J.G., Ma, T., Han, P., Fang, F., & Cao, B.Y. (2013). Combined effects of four SNPs within goat PRLR gene on milk production traits. *Gene*, 529, 276-281.

Işık, R. (2017). Saanen Keçilerinde β- Laktoglobulin (β-LG) Geni Ekzon 7 Polimorfizmi ve Süt Verimi ile İlişkisi. *Tekirdağ Ziraat Fakültesi Dergisi*, 35-40.

Kersten, S. (2014). Integrated physiology and systems biology of PPARα. *Molecular Metabolism*, 3 (4): 354-371.

Lee, J.Y., Ha, J.J., Park, Y.S., Yi, J.K., Lee, S., Mun, S., Han, K., Kim, J.J., Kim, H.J., & Oh, D.Y. (2016). Relationship between single nucleotide polymorphisms in the peroxisome proliferator-activated receptor gamma gene and fatty acid composition in Korean native cattle. *Asian-Australasian Journal of Animal Sciences*, 29 pp. 184-194.

Ferreira, L., Soares, M.A.M., Rodrigues, M.T., de Araujo, J.L.S., de Melo, A.L.P., Gasparino, E., Garcia, & O.S.R. (2020). *UCP2* and *PPARG* gene polymorphisms and their association with milk yield and composition traits in goats. *Small Ruminant Research*, Volume 192, 106210.

Martin, H. (2010). Role of PPAR-gamma in inflammation. Prospects for therapeutic intervention by food components. *Mutation Research*, 690 (1–2): 57-63.

McKenna, N.J., & O'Malley, B.W. (2002). Combinatorial control of gene expression by nuclear receptors and coregulators. *Cell*, vol. 108, no. 4, pp. 465-474.

Najafi, M.N.., Mortazavi, S.A., Koocheki, A., Khorami, J., & Rekik, B. (2009). Fat and protein contents, acidity and somatic cell counts in bulk milk of holstein cows in the Khorasan Razavi province, Iran. *International Journal of Dairy Technology*, 62(1): 19-26.

Pramod, R.K., Velayutham, D., Zachariah, A., Zachariah, A., Santhosh, S., Iype, S., Dhinoth Kumar, B., Gupta, R., & Thomas, G. (2018). The complete mitochondrial genome of Indian cattle [Bos indicus]. *Mitochondrial DNA Part B.* 3: 207–208.

Shi, H.B., Luo, J., Yao, D.W., Zhu, J.J., Xu, H.F., Shi, H.P., & Loor J.J. (2013a). Peroxisome proliferator-activated receptor-Y stimulates the synthesis of monounsaturated fatty acids in dairy goat mammary epithelial cells via the control of stearoyl-coenzyme A desaturase. *Journal of Dairy Science*, 96:7844-7853.

Shi, H., Luo, J., Zhu, J., Li, J., Sun, Y., Lin, X., Zhang, L., Yao, D., & Shi, H. (2013b). PPAR γ regulates genes involved in triacylglycerol synthesis and secretion in mammary gland epithelial cells of dairy goats. *PPAR research*, 2013: 310948. https://doi.org/10.1155/2013/310948.

Shipman, K.E., Strange, R.C., & Ramachandran, S. (2016). Use of fibrates in the metabolic syndrome: A review. *World Journal of Diabetes*, 7: 74–88.

Somacount150 Operator's Manual. (1998). *A Guide for installation, operation, and maintenance*. Bentley Instruments, Inc. Revised edition. Printed in the USA.

Yakan, A., Özkan, H., Eraslan, Ş., Ünal, N., & Özbeyaz, C. (2018). Gene expression levels in some candidate genes for mastitis resistance, milk yield, and milk quality of goats reared under different feeding systems. *Turkish Journal of Veterinary and Animal Sciences*, 42: 18-28.