

## DETERMINATION EFFECTS OF SLC27A3 AND $\beta$ -LACTOGLOBULIN GENE POLYMORPHISMS ON THE MILK COMPOSITION IN HAMDANI SHEEP

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**Abstract.** SLC27A3 and  $\beta$ -Lactoglobulin are candidate genes that affect milk traits. This study aimed to determine the effect of polymorphisms of the SLC27A3 and  $\beta$ -Lactoglobulin genes on Hamdani sheep's milk components. The PCR-RFLP method was used to detect the SLC27A3 polymorphisms at exon 2, exon 3, and exon 4. The allele frequency of SNP1, SNP2 and SNP3 were 0.59(G), 0.41(T); 0.57(G), 0.43(C) and 0.58(A) and 0.42(C) respectively.  $\beta$ -lactoglobulin allele frequency was 0.56(A) and 0.44(B). The statistical analysis showed an association between SLC27A3 and  $\beta$ -lactoglobulin polymorphisms and milk composition. The SLC27A3 and  $\beta$ -lactoglobulin polymorphisms can be used as a genetic marker to select milk component traits in sheep breeding programs.

**Keywords:** *native breed, candidate gene, allele frequency, association analysis, SNP*

### Introduction

Milk is a complete diet; it contains essential nutrients that the body needs to perform vital functions. Many factors affect milk yield, the most important of which are genes and the environment (Aytekin and Boztepe, 2013; Giambra et al., 2014; Şahin et al., 2018). Milk is also the main ingredient for dairy products (Caja and Bocquier, 2000; Moatsou and Sakkas, 2019; Rachagani and Gupta, 2008). Cows occupy the first place in providing milk, but in countries that lack rich lands, sheep and goats remain essential sources of milk production (Kalyankar et al., 2016; Raynal-Ljutovac et al., 2008). Iraqi sheep belong to the fat-tailed Asian sheep and include three breeds; Karadi, Arabi and Awassi. Karadi sheep are white, while the head and shoulders are covered with black or dark brown wool (Al-Barzinji et al., 2011; Aziz and Oramary, 2005). Two distinct breeds are belonging to Karadi: immigrant, Al-Harkia and Al-Jaf, and a non-migratory breed, which is the Dzdia and Hamdani. The Hamdani sheep is one of the largest breeds in size, as the weight of the ram is about 85 - 90 kg, and the ewe is 75 - 80 kg. Its reproductive efficiency reaches 105% and produces milk and meat (Al-Barzinji and Othman, 2013; Alkass et al., 2021).

Increasing Hamdani sheep's productivity requires genetic improvement, such as determining candidate genes that affect milk traits. Many candidate genes affect production traits in sheep, among which are the SLC27A3 and  $\beta$ -lactoglobulin genes.

Fatty acid transport proteins (FATPs or SLC27A), also known as a solute carrier protein family 27 (SLC27), are transporter families, allowing the absorption of the fatty acid into the cells. This subfamily is part of the solute carrier protein family. This family contains six very homologous proteins highly conserved during evolution and expressed in all body tissues that use fatty acids (Doege and Stahl, 2006; Gimeno,

2007; Houten and Wanders, 2010). The six FATPs are found in various tissues: FATP1 (SLC27A1): brown and white adipose tissue; FATP2 (SLC27A2): liver, kidney cortex; FATP3 (SLC27A3): fats, lungs; FATP4 (SLC27A4): liver, kidneys; FATP5 (SLC27A5): basal membrane of hepatocytes in the liver; FATP6 (SLC27A6): heart and skeletal muscle (Anderson and Stahl, 2013; Stahl, 2004; Stahl et al., 2001). SLC27A3 (Solute carrier family 27 member 3) encodes a protein involved in lipid metabolism. The increased expression of this gene in human neural stem cells derived from induced pluripotent stem cells suggests that it plays an essential role in early brain development (Hirschmugl et al., 2017; Pei et al., 2009; Sun et al., 2014). Ovine SLC27A3 gene located at chromosome 1, contains 10 exons and encodes 680 amino acids (Pecka-Kielb et al., 2020). SLC27A3 gene is considered a candidate gene for milk traits in sheep (Pecka-Kielb et al., 2020; Calvo et al., 2006; Kulig et al., 2010; Kowalewska-Luczak et al., 2017). Casein constitute about 80% of milk proteins and consists of;  $\alpha$ s1-casein (CSN1S1),  $\alpha$ s2-casein (CSN1S2),  $\beta$ -casein (CSN2) and  $\kappa$ -casein (CSN3). Whey proteins constitute about 20% of milk proteins and consist of;  $\alpha$ -lactalbumin,  $\beta$ -lactoglobulin, and other proteins (Čítek et al., 2019; Moiola et al., 2007).  $\beta$ -lactoglobulin is the major whey protein of sheep milk, and it is also present in many other mammalian species, but it is absent in human milk (Gras et al., 2016; Masala et al., 2019).  $\beta$ -lactoglobulin found it associated with milk yield, composition and cheese making. The molecular weight of  $\beta$ -lactoglobulin is 36.4 kDa and consists of 162 amino acids (Elmaci et al., 2006; Triantaphyllopoulos et al., 2017). Ovine  $\beta$ -lactoglobulin located at chromosome 3, and three different variants have been determined; A, B, and C. In cattle, detected fifteen polymorphic variants of  $\beta$ -lactoglobulin gene out of which two variants A and B are most frequent (Elmaci et al., 2007; El-Shazly et al., 2012). The A variants (tyrosine) and B (histidine) differ at the amino acid position 20. An association has been described between  $\beta$ -lactoglobulin polymorphism and sheep milk composition (Othman et al., 2012; Rashaydeh et al., 2020).

This study explores to find the association between the SLC27A3 and  $\beta$ -lactoglobulin genes polymorphism and milk components in Hamdani sheep.

## Materials and methods

### *Experimental animals*

A total of 60 ewes of Hamdani sheep were used in the study. The ewes were three years old and taken from commercial farms in Kirkuk city/Iraq.

Milk samples were taken twice a month for three months. Ewes were fed on a ration consisting of 55% barley, 33% of wheat bran, 10% of soybean meal, 1% of salt, and 1% of lime, with two morning and evening meals at a rate of 500 gm/head/day with continuous water provision in addition to mineral salts, and hay was provided at a rate of 500 gm/head/day.

### *Milk components analysis*

Milk components were measured twice a month for each ewe for three months. Milk samples were taken in the morning and the milk was placed in 60 mm plastic containers. Milk components (fat, protein, lactose, non-fat solids, and total solids) were analyzed by Milkotester Master Classic LM2.

### Sample collection and DNA extraction

Genetic analysis was carried out in the molecular genetics laboratory at the College of Veterinary, University of Kirkuk. The blood was collected from the jugular vein using tubes containing ethylenediamine tetra-acetic acid (EDTA) and stored at -20 °C. Genomic DNA was extracted from whole blood by using the phenol-chloroform methods. The primer sequence of the SLC27A3 gene locus was shown in *Table 1*. The PCR was done in a reaction volume of 20  $\mu$ L, contains 5  $\mu$ L (50 ng) DNA, 5  $\mu$ L of PCR Master Mix (GoTaq® G2 Green Master Mix, Promega, USA), 0.5  $\mu$ L for each primer (10  $\mu$ mol) and 9  $\mu$ L distilled water.

**Table 1.** The primer sequences of SLC27A3 and  $\beta$ -lactoglobulin gene locus

SNP	Position	Primer sequence	FL	RE	PCR conditions	Reference
SNP1	exon 2	5'-GTAGAACTGCGGGGCTGTG-3' 5'-AGGAGGTCATAGTTCCTGTTC-3'	319 bp	<i>Hpy188 III</i>	94 °C 5 m, 94 °C 30 s, 53 °C 45 s, 72 °C 45 s, 30 cycles 72 °C 5 m	Pecka-Kielb et al. (2020)
SNP2	exon 3	5'-GAGACAAGGCTTGGGTTTCAG-3' 5'-AGCCTCCTCCTCTCCATTC-3'	354 bp	<i>ScrFI</i>	94 °C 5 m, 94 °C 30 s, 53 °C 45 s, 72 °C 45 s, 30 cycles 72 °C 5 m	
SNP3	exon 4	5'-TCTGGGAAGAAGGGAGTCAG-3' 5'-TCTCCCCCTCCATTTCTT-3'	337 bp	<i>Fnu4HI</i>	94 °C 5 m, 94 °C 30 s, 50 °C 45 s, 72 °C 45 s, 30 cycles 72 °C 5 m	
$\beta$ -lactoglobulin	exon 2	5'-CAACTCAAGGTCCCTCTCCA-3' 5'-CTTCAGCTCCTCCACGTACA-3'	120 bp	<i>RsaI</i>	94 °C 5 m, 95 °C 15 s, 60 °C 30 s, 72 °C 1 m, 36 cycles 72 °C 10 m	Feligini et al. (1998)

FL: fragment length

### PCR-RFLP method

The mix consisted of 10  $\mu$ L PCR product, 4  $\mu$ L distilled water, 2  $\mu$ L 10X buffer and 1  $\mu$ L/U restriction enzyme (Total of 17  $\mu$ L). Digestion products were separated at 3% agarose gel in 80 V for 60 min. The gel stained by ethidium bromide. The results were checked under ultraviolet lights.

### Statistical analysis

The allele and genotype frequency of the genes and the Chi-square test  $\chi^2$  were calculated by popgen32 (ver.1.32). Association analyses were done by using the General Linear Model (GLM) of Minitab 16. The least-squares means were compared using Tukey, the least significant difference test.

The general linear model was:

$$Y_{ij} = \mu + \alpha_i + e_{ij}$$

where:  $Y_{ij}$ : traits measured,  $\mu$ : overall mean for each trait,  $\alpha_i$ : genotypes effect,  $e_{ij}$ : random error.

## Results

### SNP1 locus polymorphism

319 bp of PCR product was amplified. Three genotypes (GG, GT and TT) were obtained. GG genotype was 319 bp; GT genotype was 319, 194 and 125 bp; TT genotype was 194 and 125 bp (Fig. 1). Chi-square  $\chi^2$  test showed agreement to Hardy-Weinberg equilibrium ( $p > 0.05$ ) (Table 2). The allele and genotype frequency was 0.59(G) and 0.41(T); 0.38(GG), 0.42(GT) and 0.20(TT).

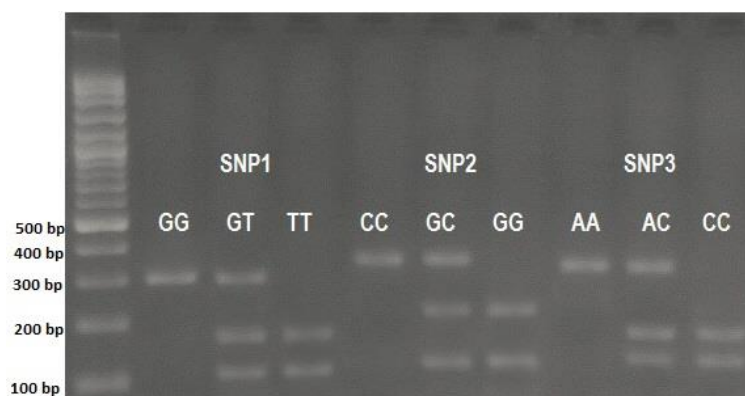


Figure 1. PCR-RFLP band patterns of the SLC27A3 gene

Table 2. Genotype and allele frequency of the SLC27A3 and  $\beta$ -lactoglobulin genes

Polymorphism	Genotype frequencies	Allele frequencies	$\chi^2$
SNP1	0.38(GG) 0.42(GT) 0.20(TT)	0.59(G) 0.41(T)	1.13
SNP2	0.36(GG) 0.42(GC) 0.22(CC)	0.57(G) 0.43(C)	1.30
SNP3	0.36(AA) 0.43(AC) 0.20(CC)	0.58(A) 0.42(C)	0.70
$\beta$ -lactoglobulin	0.28(AA) 0.55(AB) 0.17(BB)	0.56(A) 0.44(B)	0.79

### SNP2 locus polymorphism

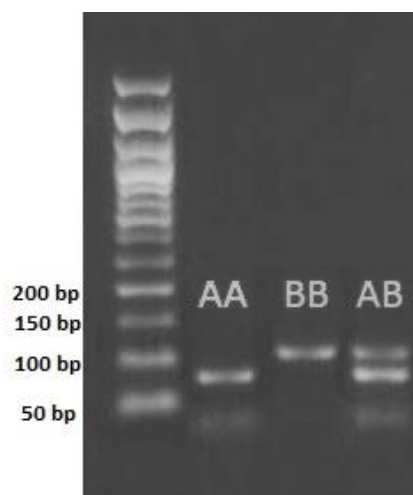
354 bp of PCR product was amplified. Three genotypes (GG, GC and CC) were obtained. GG genotype was 222 and 132 bp; GC genotype was 354, 222 and 132 bp; CC genotype was 354 bp (Fig. 1). Chi-square  $\chi^2$  test showed agreement to Hardy-Weinberg equilibrium ( $p > 0.05$ ) (Table 2). The allele and genotype frequency was 0.57(G) and 0.43(C); 0.36(GG), 0.42(GC) and 0.22(CC).

### ***SNP3 locus polymorphism***

337 bp of PCR product was amplified. Three genotypes (AA, AC and CC) were obtained. AA genotype was 337 bp; AC genotype was 337, 190 and 147 bp; CC genotype was 190 and 147 bp (*Fig. 1*). Chi-square  $\chi^2$  test showed agreement to Hardy-Weinberg equilibrium ( $p > 0.05$ ) (*Table 2*). The allele and genotype frequency was 0.58(A) and 0.42(C); 0.36(AA), 0.43(AC) and 0.20(CC).

### ***$\beta$ -Lactoglobulin locus polymorphism***

120 bp of PCR product was amplified. Three genotypes (AA, AB and BB) were determined. AA genotype was 66, 37 and 17 bp; AB genotype was 103, 66, 37 and 17 bp; BB genotype was 103 and 17 bp (*Fig. 2*). Chi-square  $\chi^2$  test showed agreement to Hardy-Weinberg equilibrium ( $p > 0.05$ ) (*Table 2*). The allele and genotype frequency was 0.56(A) and 0.44(B); 0.28(AA), 0.55(AB) and 0.17(BB).



**Figure 2.** PCR-RFLP band patterns of the  $\beta$ -lactoglobulin gene

### **Discussion**

In this study, three genotypes were identified of SNP1, SNP2 and SNP3. The allele frequency was 0.59(G), 0.41(T) of SNP1, 0.57(G), 0.43(C) of SNP2 and 0.58(A), 0.42(C) of SNP3. Also, Pecka-Kielb et al. (2020) showed allele frequency in Zosl ahtena valaska sheep for SNP1, SNP2 and SNP3 as 0.53(G), 0.47(T); 0.53(G), 0.47(C); 0.55(A), 0.45(C) respectively. The results show a similarity with a slight increase of allele frequency in Hamdani sheep. The association results showed that there were significant association between the SLC27A3 gene polymorphisms and fat, protein and total solids ( $p < 0.05$ ) (*Table 3*). The SNP1 polymorphisms were associated with fat, protein and total solids. SNP2 polymorphism showed an association only with protein. Whereas the SNP3 did not show any association between the polymorphism and milk composition. Also, Pecka-Kielb et al. (2020) did not find any association between SNP2 and milk composition. The TT genotypes of SNP1 showed the lowest fat content ( $4.466 \pm 0.158$ ) compared to other genotypes.

Pecka-Kielb et al. (2020) found influence of the SLC27A3 gene polymorphisms on the fat, lactose and dry matter content in sheep milk. Calvo et al. (2006) determined SLC27A3 as candidate genes for fat content in sheep milk.

**Table 3.** Association analysis between SLC27A3 gene locus and milk composition

Traits	Genotypes (mean $\pm$ standard error)			p-value
	SNP1			
	GG	GT	TT	
Fat %	4.890 $\pm$ 0.112 <sup>ab</sup>	5.028 $\pm$ 0.107 <sup>a</sup>	4.466 $\pm$ 0.158 <sup>b</sup>	<b>0.014</b>
Protein %	6.584 $\pm$ 0.029 <sup>ab</sup>	6.570 $\pm$ 0.028 <sup>b</sup>	6.695 $\pm$ 0.041 <sup>a</sup>	<b>0.037</b>
Lactose %	4.458 $\pm$ 0.034	4.372 $\pm$ 0.032	4.395 $\pm$ 0.048	0.195
Non-fat solids %	11.79 $\pm$ 0.118	11.89 $\pm$ 0.112	11.85 $\pm$ 0.166	0.826
Total solids %	15.46 $\pm$ 0.045 <sup>a</sup>	15.32 $\pm$ 0.043 <sup>ab</sup>	15.24 $\pm$ 0.063 <sup>b</sup>	<b>0.016</b>
	SNP2			
	GG	GC	CC	
Fat %	4.706 $\pm$ 0.120	4.709 $\pm$ 0.113	4.969 $\pm$ 0.140	0.296
Protein %	6.687 $\pm$ 0.031 <sup>a</sup>	6.599 $\pm$ 0.029 <sup>ab</sup>	6.564 $\pm$ 0.036 <sup>b</sup>	<b>0.029</b>
Lactose %	4.353 $\pm$ 0.036	4.460 $\pm$ 0.034	4.412 $\pm$ 0.042	0.681
Non-fat solids %	11.73 $\pm$ 0.126	12.04 $\pm$ 0.118	11.75 $\pm$ 0.147	0.118
Total solids %	15.33 $\pm$ 0.048	15.35 $\pm$ 0.045	15.34 $\pm$ 0.056	0.942
	SNP3			
	AA	AC	CC	
Fat %	5.017 $\pm$ 0.116	4.665 $\pm$ 0.107	4.703 $\pm$ 0.130	0.066
Protein %	6.647 $\pm$ 0.030	6.613 $\pm$ 0.028	6.590 $\pm$ 0.034	0.445
Lactose %	4.394 $\pm$ 0.035	4.398 $\pm$ 0.032	4.434 $\pm$ 0.039	0.681
Non-fat solids %	11.92 $\pm$ 0.121	11.79 $\pm$ 0.112	11.81 $\pm$ 0.137	0.706
Total solids %	15.29 $\pm$ 0.046	15.36 $\pm$ 0.043	15.37 $\pm$ 0.052	0.434
	$\beta$ -Lactoglobulin			
	AA	AB	BB	
Fat %	4.759 $\pm$ 0.104 <sup>ab</sup>	5.047 $\pm$ 0.117 <sup>a</sup>	4.578 $\pm$ 0.152 <sup>b</sup>	<b>0.040</b>
Protein %	6.606 $\pm$ 0.027	6.678 $\pm$ 0.030	6.566 $\pm$ 0.040	0.065
Lactose %	4.349 $\pm$ 0.031 <sup>b</sup>	4.374 $\pm$ 0.035 <sup>ab</sup>	4.502 $\pm$ 0.046 <sup>a</sup>	<b>0.021</b>
Non-fat solids %	11.82 $\pm$ 0.110	12.01 $\pm$ 0.123	11.70 $\pm$ 0.159	0.267
Total solids %	15.38 $\pm$ 0.042	15.31 $\pm$ 0.047	15.34 $\pm$ 0.061	0.555

<sup>a,b</sup>There are significant differences ( $p < 0.05$ )

The allele frequency of  $\beta$ -lactoglobulin was 0.56(A) and 0.44(B). Various studies showed that the A allele frequency is usually higher than the B allele. The A allele frequencies were; 0.78 in the Karacabey Merino sheep (Elmaci et al., 2007); 0.68 in the Teleorman Black Head sheep (Gras et al., 2016); 0.58 in the Karagouniko sheep (Triantaphyllopoulos et al., 2017); 0.78, 0.76 and 0.98 in the Kivircik, Gökçeada and Sakız respectively (Elmaci et al., 2006); 0.55 in Pramenka breed (Rustempašić et al., 2018); 0.71 in Karakul (Kevorkian et al., 2008). However, in some studies, the B allele frequency was higher than A allele frequency: 0.53 in Kivircik (Gürcan et al., 2018); 0.52 in Dubska Pramenka (Masala et al., 2019); 0.52 in Awassi (Dakheel et al., 2021).

The statistical analysis showed the influence of  $\beta$ -lactoglobulin gene polymorphisms on fat and lactose content. The BB genotypes showed higher lactose content ( $4.578 \pm 0.152$ ) compared to other genotypes.

The A variant of  $\beta$ -lactoglobulin is associated with protein, fat casein content, and total solids. Whereas, B allele is associated with higher milk yield. The AA genotype is more efficient for cheese manufacturing than other genotypes AB and BB (Amigo et al., 2000). Significant associations were determined between  $\beta$ -lactoglobulin gene polymorphisms and protein, fat, lactose, and solids non-fat in Awassi and Morkaraman sheep breeds (Çelik and Özdemir, 2007). No association was identified between  $\beta$ -lactoglobulin variants and milk components in sheep Noami, Sawakni, Harry and Nagdi (El-Shazly et al., 2012). Gras et al. (2016) found association between  $\beta$ -lactoglobulin and milk yield and composition. Triantaphyllopoulos et al. (2017) determined significant effects of  $\beta$ -lactoglobulin polymorphism on lactose percentage and somatic cell count (SCC). The BB genotype of the  $\beta$ -lactoglobulin gene showed the highest fat, protein, solids non-fat, lactose, and density compared to other AA and BB (Jawasreh et al., 2019). The effect of the  $\beta$ -lactoglobulin gene was determined on fat content and density in Awassi sheep (Rashaydeh et al., 2020). BB genotype showed highest milk yield (Dakheel et al., 2021).

## Conclusions

The Hamdani breeds shown genetic polymorphisms of SLC27A3 and  $\beta$ -lactoglobulin gene locus. The results showed the effect of the SLC27A3 gene locus on protein, fat and total solids. Also, the  $\beta$ -lactoglobulin gene locus shown a significant association with fat and lactose. Therefore, these genes can be used as genetic markers to improve milk traits in Hamdani sheep. However, the frequency of these genes must also be increased by following appropriate breeding programs in Hamdani sheep.

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