Effects of SLC27A3 and β -Lactoglobulin Gene Polymorphisms on The Milk Composition in Hamdani Sheep

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Research

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Abstract

Background: SLC27A3 and β -Lactoglobulin gene are candidate genes that effects milk traits.

Results: This study aimed to determine the effect of polymorphisms of the SLC27A3 gene 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. β -lactoglobulin allele frequency was 0.56(A) and 0.44(B). The statistical analysis showed an association between SLC27A3 and β -lactoglobulin polymorphisms and milk composition. **Conclusions:** The SLC27A3 and β -lactoglobulin polymorphisms can be used as a genetic marker to select milk component traits in the sheep breeding programs.

Background

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 et al. 2013; Giambra et al. 2014; Şahin et al. 2018). Milk is also the main ingredient for dairy products (Caja et al. 2000; Moatsou et al. 2019; Rachagani et al. 2008). Cows occupy the first place in securing countries with milk, but in countries that lack rich lands, sheep and goats remain essential sources of securing milk (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 et al. 2005). There are two distinct breeds belong to Karadi, the first: immigrant, which is 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 is used to produce milk and meat (Al-Barzinji et al. 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 β -lactoglobulin genes.

Fatty acid transport proteins (FATPs or SLC27A), also known as solute carrier protein family 27 (SLC27), are transporter families, allows 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 et al. 2006; Gimeno, 2007; Houten et al. 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 et al. 2013; Stahl, 2004; Stahl et al. 2001). SLC27A3 (Solute carrier family 27 member 3) this gene 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-Kiełb et al. 2020). SLC27A3 gene is considered a candidate gene for milk traits in sheep (Calvo et al. 2006; Kowalewska-Łuczak et al. 2017; Kulig et al. 2010; Pecka-Kiełb et al. 2020). Casein constitute

about 80% of milk proteins and consists of; α s1-casein (CSN1S1), α s2-casein (CSN1S2), β -casein (CSN2) and κ -casein (CSN3). Whey proteins constitute about 20% of milk proteins and consist of; α -lactalbumin, β -lactoglobulin, and other proteins (Čítek et al. 2019; Moiola et al. 2007). β -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). β -lactoglobulin found it associated with milk yield, composition and cheese making. The molecular weight of β -lactoglobulin is 36.4 kDa and consists of 162 amino acids (Elmaci et al. 2006; Triantaphyllopoulos et al. 2017). Ovine β -lactoglobulin located at chromosome 3, and three different variants have been determined; A, B, and C. In cattle, detected fifteen polymorphic variants of β -lactoglobulin gene out of which two variants A and B are most frequent (El-Shazly et al. 2012; Elmaci et al. 2007). The A variants (tyrosine) and B (histidine) differ at the amino acid position 20. An association has been described between β -lactoglobulin polymorphism and sheep milk composition (Othman et al. 2012; Rashaydeh et al. 2020).

This study aims to find the association between the SLC27A3 and β -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. Milk samples were taken twice a month for three months.

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 μ L, contains 5 μ L (50 ng) DNA, 5 μ L of PCR Master Mix (GoTaq® G2 Green Master Mix, Promega, USA), 0.5 μ L for each primer (10 μ mol) and 9 μ L distilled water.

PCR-RFLP method

The mix consisted of 10 μ L PCR product, 4 μ L distilled water, 2 μ L 10X buffer and 1 μ L restriction enzyme (Total of 17 μ L). Digestion products were separated at 3% agarose gel in 80 V for 60 min. The gel stained by ethidium bromide and used a 1000 bp DNA marker (Promega, USA). The results were checked under ultraviolet lights.

Statistical Analysis

The allele and genotype frequency of the genes and the Chi-square test χ^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:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + S_k + e_{ijk}$$

Yijk: traits measured

μ : overall mean for each trait

α_i : genotypes effect

β_j : farms effect (1, 2)

e_{ijk} : 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 194 bp; TT genotype was 125 bp. Chi-square χ^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).

SNP2 locus polymorphism

354 bp of PCR product was amplified. Three genotypes (GG, GC and CC) were obtained. GG genotype was 354 bp; GC genotype was 222 bp; CC genotype was 132 bp. Chi-square χ^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 190 bp; CC genotype was 147 bp. Chi-square χ^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).

β -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. Chi-square χ^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).

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-Kiełb et al. (2020) showed allele frequency in Zosł achtena 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 (Table 3). The SNP1 polymorphisms were associated with fat, protein and total solids. SNP2 polymorphism showed an association only with protein. Also, SNP3 was associated only with fat. The GT and AA genotypes had the highest fat content (5.028 ± 0.107) and (5.017 ± 0.116) respectively, compared to other genotypes. Pecka-Kiełb 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.

The allele frequency of β -lactoglobulin was 0.56(A) and 0.44(B). Various studies showed that the A allele frequency is usually higher than the B allele: 0.78 in the Karacabey Merino sheep (Elmaci et al. 2006); 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. The association results showed that there were significant association between the β -lactoglobulin gene polymorphisms and fat, protein and lactose. Gras et al. (2016) found association between β -lactoglobulin and milk yield and composition. Triantaphyllopoulos et al. (2017) determined significant effects of β -lactoglobulin polymorphism on lactose percentage and somatic cell count (SCC).

Conclusions

There are a few studies that explain gene polymorphism and determine the gene's association with milk traits. In this study, we determined the association of SLC27A3 and β -lactoglobulin gen polymorphisms with milk components. The gene can be used as a candidate gene to improve milk traits. However, further studies are required in different sheep breeds.

Abbreviations

SLC27: solute carrier protein family 27, SNP: Single nucleotide polymorphism

Declarations

Acknowledgements

None.

Authors' contributions

MB conceptualized and designed the study, OS organized and performed the samplings, performed the analysis.

Ethics approval

No. 141/44107.

Availability of data and materials

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 The primer sequences of SLC27A3 and β -lactoglobulin gene locus

SNP	Position	Primer Sequence	FL	RE	PCR conditions	Reference
SNP1	exon 2	5'-GTAGAACTGCGGGGCTGTG-3' 5'-AGGAGGTCATAGTTCCTGTTCC-3'	319bp	<i>Hpy188 III</i>	94°C 5m, 94°C 30s, 53°C 45s, 72°C 45s, 30 cycles 72°C 5m	Pecka-Kiełb et al. (2020)
SNP2	exon 3	5'-GAGACAAGGCTTGGGTTCAG-3' 5'-AGCCTCCTTCCTCTCCATTC-3'	354bp	<i>ScrFI</i>	94°C 5m, 94°C 30s, 53°C 45s, 72°C 45s, 30 cycles 72°C 5m	
SNP3	exon 4	5'-TCTGGGAAGAAGGGAGTCAG-3' 5'-TCTCCCCCTTCCATTTTCTT-3'	337bp	<i>Fnu4HI</i>	94°C 5m, 94°C 30s, 50°C 45s, 72°C 45s, 30 cycles 72°C 5m	
β -lactoglobulin	exon 2	5'-CAACTCAAGGTCCTCTCCA-3' 5'-CTTCAGCTCCTCCACGTACA-3'	120bp	<i>RsaI</i>	94°C 5m, 95°C 15s, 60°C 30s, 72°C 1m, 36 cycles 72°C 10m	Feligini et al. (1998)

FL: fragment length

Table 2 genotype and allele frequency of the SLC27A3 and β -lactoglobulin genes

Polymorphism	n	Genotype Frequencies	Allele Frequencies	χ^2
SNP1	23	0.38(GG)	0.59(G)	1.13
	25	0.42(GT)	0.41(T)	
	12	0.20(TT)		
SNP2	22	0.36(GG)	0.57(G)	1.30
	25	0.42(GC)	0.43(C)	
	13	0.22(CC)		
SNP3	22	0.36(AA)	0.58(A)	0.70
	26	0.43(AC)	0.42(C)	
	12	0.20(CC)		
β -lactoglobulin	17	0.28(AA)	0.56(A)	0.79
	33	0.55(AB)	0.44(B)	
	10	0.17(BB)		

Table 3 Association analysis between SLC27A3 gene locus and milk composition

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

* (p<0.05); ** (p<0.10)