PAEP gene restriction fragment length polymorphism and its effects on milk composition in cross-bred Hamdani sheep

ABSTRACT

Beta-lactoglobulin (βLG) stands as the primary whey protein in ruminant milk, synthesized by mammary gland cells during lactation and encoded by the progestagen-associated endometrial protein (*PAEP)* gene. This study aimed to assess the impact of *PAEP* gene exon II polymorphism on milk composition traits in cross-bred Hamdani sheep. Sheep were examined for clinical diseases and mastitis. Milk and blood samples were only collected from healthy ewes. The composition and physical properties of milk were analyzed using milk autoanalyzer. The *PAEP* gene exon II region's 452 bp PCR products were subjected to restriction fragment length polymorphism (RFLP) analysis using the *RsaI* restriction enzyme. Two genotypes, AA and AB, were identified for the *PAEP* gene exon II region, with A and B allele frequencies of 0.7 and 0.3, respectively. Statistical analysis, conducted with Minitab® (Version: 19.2020.2.0), revealed that the AA genotype is associated with a higher milk fat percentage ($p<0.05$). However, no significant genotype effect was observed for other milk composition traits in cross-bred Hamdani sheep. These results suggest that *PAEP* genotypes could serve as valuable indicators for enhancing milk composition in cross-bred Hamdani sheep through breeding programs.

Keywords: Beta-lactoglobulin, cross-bred Hamdani sheep, milk fat, *PAEP*, PCR-RFLP

INTRODUCTION

Sheep milk serves as a significant source of income for rural breeders worldwide. In comparison to cow milk, sheep milk boasts superior nutritional value, with higher fat and protein content crucial for milk products (Wendorff and Haenlein, 2017). Several factors, including lactation stage, management, diseases, and genetic factors, can influence milk production and composition in sheep (Koca et al., 2023; Komprej et al., 2012). The primary components of sheep milk include fat, protein, lactose, and minerals. Numerous studies indicate a strong correlation between milk composition and gene polymorphism in sheep, revealing various genetic variants on milk-related genes (Kusza et al., 2018; Özmen and Kul, 2016; Selvaggi et al., 2014; Yousefi et al., 2013).

Beta-lactoglobulin (βLG), the primary whey protein in ruminant milk, is produced by mammary gland secretory cells during lactation and is encoded by the progestagen-associated endometrial protein (*PAEP)* gene located on chromosome 3, consisting of seven protein-coding exons (Feligini et al., 1998). Polymorphic *PAEP* variants' effects on milk composition have been reported in different sheep breeds, including the

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Research Article

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A, B, and C variants (Kusza et al., 2018; Rustempašić et al., 2018). The genetic variations A and B show a disparity in amino acid position 20, with variant A featuring a histidine instead of threonine found in variant B (Kolde et al., 1983; Anton et al., 1999). Additionally, variant C, a subtype of variant A, involves a sole amino acid substitution, changing arginine to glutamic acid at position 148 (Erhardt, 1989; Anton et al., 1999). While A and B variants are prevalent across all breeds, the C variant is a rare allele specifically identified in the Merino breed (Kusza et al., 2018). Notably, the A variant of *PAEP* is linked to fat and protein, whereas the B variant shows an association with increased milk yield (Selvaggi et al., 2014).

The sheep industry plays a crucial role in Türkiye, contributing significantly to animal products. According to recent data, sheep milk production reached 1.067 million tonnes in 2022, positioning Türkiye as a leading country in sheep milk production (TUIK, 2022). However, limited use of artificial insemination and milking record systems due to traditional breeding practices restricts genetic improvements in sheep milk traits. These limitations underscore the importance of DNA markers influencing milk traits in sheep (Staiger et al., 2010). Cross-bred Hamdani sheep, primarily bred in the Siirt region and its surroundings, exhibit higher fat content compared to other sheep breeds in Türkiye (Turgut et al., 2023). This study aims to identify *PAEP* gene exon II polymorphisms and their associations with milk composition traits in cross-bred Hamdani sheep raised in the Southeastern Anatolia Region of Türkiye.

MATERIALS AND METHODS

Animals and sample collection

In the study, a total of 96 cross-bred Hamdani ewes were used. Animals were fed on pasture and 250 g of barley and 400 g of hay were added to their rations. Blood samples were taken from the *vena jugularis* in 9 mL K_3EDTA-containing tubes (BD Vacutainer®, Becton Dickinson, Türkiye). Blood samples were mixed slightly and stored at -20°C until further examination. Milk samples were collected in sterile 50 mL falcon tubes and transferred to the lab on the ice blocks immediately. Milk samples were collected before the morning and evening milking routine. Sheep were examined for clinical diseases, clinical and subclinical mastitis. And samples were only collected from healthy ewes at 2-3 ages during the spring season. Body condition scores (BCS) of ewes were between 2.75-3. BCS was evaluated according to Russel et al. (1969). All animals were 25-40 days of lactation that represents early lactation stage.

Milk composition analysis

The milk samples underwent analysis using an ultrasonic milk analyzer, specifically the Lactoscan® SA Milk Analyzer (Milkotronic Ltd, Nova Zagora, Bulgaria). The device was calibrated for sheep milk. For each analysis, a total of 15 mL of milk sample was employed, and various parameters were recorded, including milk fat (%), solids-not-fat (SNF) (%), milk protein (%), lactose (%), pH, salt (%), and density (kg/m^3). To ensure the accuracy of the results, the outputs of the milk autoanalyzer were subjected to verification using methods outlined by the Association of Official Analytical Chemists (AOAC). The AOAC Official Method 925.23 was employed for determining total solids in milk. Protein percentage was ascertained using the AOAC official method, known as Kjeldahl's method (Barbano et al., 1990). The fat percentage was assessed using the Gerber method (Kleyn et al., 2001). This dual verification approach, involving both the autoanalyzer and AOAC methods, ensured the reliability and accuracy of the milk composition data. Then, the data obtained from the milk autoanalyzer were utilized for subsequent statistical analyses.

DNA extraction and DNA quality control

Genomic DNA was extracted from blood using a genomic DNA isolation kit (Hibrigen, Hydra Biotechnology, Türkiye) according to the

manufacturer's instruction. The purification and quantity of genomic DNA were evaluated according to optical density at 260/280 nm on a spectrophotometer (Allsheng, Hangzhou, China). DNA integrity was evaluated on %0.8 agarose gel electrophoresis.

Polymerase chain reaction (PCR)

PCR reactions were carried out in 25 µL of total volume; 50-100 ng genomic DNA, 12.5 mL PCR 2X Taq Master Mix (Hibrigen, Hydra Biotechnology, Türkiye), 5 pmol of each primer, and water up to 25 µL. The primer pairs are presented in Table 1.

Table 1. Primers and PCR product size

Gene	Region	Primers (5'→3')	Product size (bp)	References
PAEP	Exon II	F: TTGGGTTCAGTGTGAGTCTGG R: AAAAGCCCTGGGTGGGCAGC	452	Eignatev, (1998)

PCR conditions were as follows; initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 62.2°C for 30 sec, extension at 72°C for 30 sec and a final extension at 72°C for 7 min on Kyratec SC300G thermal cycler (Kyratec, Queensland, Australia). Following the reaction, 452 bp PCR products of the *PAEP* gene were visualized in %2 agarose gel stained with SYBR Safe (Hibrigen, Hydra Biotechnology, Türkiye) under UV light (Figure 1).

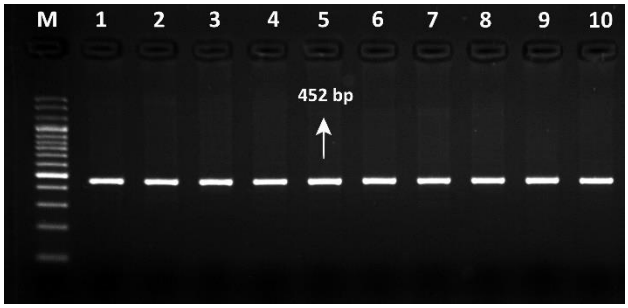

Figure 1. Results of PCR reactions. Line 1-10: 452 bp PCR products of the PAEP gene. M: 100 bp marker.

Genotyping

PAEP genotypes were identified using the restriction fragment length polymorphism (RFLP) method. RFLP reaction was carried out for 15 min at 37 °C in 50 µL of total volume; 1 µg PCR product, 10 units *RsaI* restriction enzyme (NEB, UK), 5 µL 10X rCutSmart buffer (NEB, UK), and water up to 50 µL. Then fragments were analyzed in 4% agarose gel stained with SYBR Safe (Hibrigen, Hydra Biotechnology, Türkiye) under UV light and *PAEP* genotypes were identified.

Statistical Analysis

Statistical analysis was carried out using Minitab® (Version: 19.2020.2.0, Minitab Inc., State College, PA, USA). Allel and genotype frequencies were calculated using Falconer and Mackay (1996) model. The chi-square test was used to evaluate Hardy-Weinberg Equilibrium (HWE) of alleles. Anderson-Darling normality test was applied. Due to normal distribution of the data, independent sample t-test was performed to detect the effects of *PAEP* genotypes on milk composition. The statistical significance level was defined as 0.05.

RESULTS

Allel and genotypes

Following RFLP, allele discrimination was carried out according to fragment size. In cross-bred Hamdani sheep only AA (175, 170, 66, and 41 bp) and AB (236, 175, 170, 66, and 41 bp) genotypes were identified (Figure 2). Allele and genotype frequencies are present in Table 2. Genotype frequencies for AA and AB were 0.403 and 0.597 respectively. Regarding genotypes, A allele frequency (0.7) was higher than B allel frequency (0.3). Allel distribution of *PAEP* was not in HWE ($p<0.05$).

Table 2. Allel and genotype frequencies of *PAEP* gene

		Allele Frequency		Genotypes (Observed)					
PAEP	n	A allele	B allele	AA	AB	BB	Ho	He	p-value
	96	0.7	0.3	38	58	-	0.597	0.421	**0.005**

Ho: Observed heterozygosity, He: Expected heterozygosity

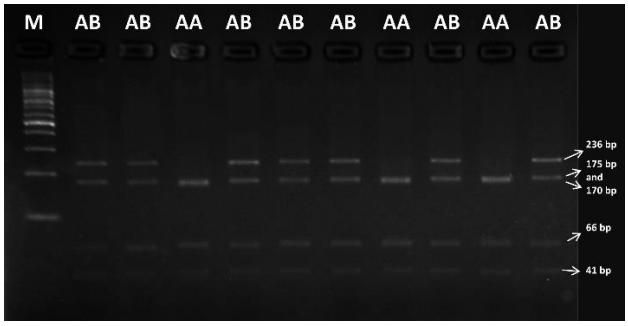

Figure 2. RsaI restriction fragments of 452 bp PCR products of PAEP gene. AA and AB genotypes. M: 100 bp DNA marker

The effects of PAEP genotypes on milk composition

The effects of AA and AB genotypes on milk composition traits are summarized in Table 3. Milk fat content (%) was higher in the AA genotype than in the AB genotype ($p<0.05$). However, there were no significant differences in protein (%), lactose (%), SNF (%), salt (%), pH, and density between AA and AB genotypes.

Table 3. The effects of *PAEP* gene AA and AB genotypes on milk composition (Mean±SE).

	***PAEP* Genotype**			
	AA	**AB**	**BB**	**p-value**
Fat (%)	7.81 ± 0.27	7.07 ± 0.17	-	**0.023**
Protein (%)	4.18 ± 0.06	4.14 ± 0.04	-	>0.05
Lactose (%)	3.94 ± 0.06	3.90 ± 0.04	-	>0.05
SNF (%)	8.78 ± 0.14	8.72 ± 0.10	-	>0.05
pH	6.86 ± 0.03	6.93 ± 0.07	-	>0.05
Density (kg/m^3)	1.0281± 0.0006	1.0283 ± 0.0004	-	>0.05
Salt (%)	0.62 ± 0.12	0.62 ± 0.01	-	>0.05

SE: Standard error, SNF: Solids-non-fat

DISCUSSION

In this study, AA and AB genotypes were identified in the *PAEP* gene exon II region of cross-bred Hamdani sheep. The A allele frequency (0.7) of the *PAEP* gene was greater than the B allele frequency (0.3). Similarly, Özmen and Kul (2016) detected only AA and AB genotypes in Sakız sheep. Moreover, A allele frequency of the *PAEP* gene were higher than B allele frequency in Sakız, Akkaraman, and Awassi sheep, consistent with the data presented in our study (Özmen and Kul, 2016). Comparable results were observed in various sheep breeds, including Egyptian (Othman et al., 2015), Latvian Darkhead (Stambekov et al., 1997), Polish (Kawecka and Radko, 2011), Racka (Barayni et al., 2010), Karagouniko (Triantaphyllopoulus et al., 2017), and Sora (Đokić et al., 2019) and Hamdani (Bayraktar and Shoshin, 2021) sheep breeds. However, Yousefi et al. (2013) reported a contrasting observation, noting that the B allele frequency of the *PAEP* gene exon II was greater than the A allele frequency in Zel sheep. Additionally, studies by Mohammadi et al. (2006), Mele et al. (2007), Dario et al. (2008), Michalcova and Krupova (2009), Corral et al. (2010), and Barayni et al. (2010) reported higher B allele frequencies than A allele frequencies in different sheep breeds. Conversely, Kusza et al. (2018) highlighted variations in A and B allele distribution of the *PAEP* gene in diverse sheep breeds reared in Eastern Europe.

Statistical analysis uncovered a significant association between the AA genotype of the *PAEP* gene and a higher fat percentage in cross-

bred Hamdani sheep. In line with our findings, Özmen and Kul (2016) reported higher milk fat percentages in AA genotypes compared to AB genotypes. In addition, Bayraktar and Shoshin, (2021) reported that milk fat percentage was higher in AA and AB genotypes compared to BB genotype while milk lactose percentage was higher in BB genotype. Fadhil and Dakheel (2022) also observed higher milk fat percentages in the AA genotype compared to the BB genotype. Conversely, Yousefi et al. (2013) noted that ewes with AB genotypes exhibited higher milk fat and lactose percentages in indigenous Zel sheep compared to ewes with the AA genotype. Giambra et al. (2014) similarly found that AB genotypes were associated with fat and lactose percentages in East Frisian dairy sheep.

No significant relationships were observed between *PAEP* genotypes and other milk composition traits in cross-bred Hamdani sheep, consistent with findings in other studies (Kawecka and Radko, 2011; Michalcova and Krupova, 2009; Sumantri et al., 2008). However, Triantaphyllopoulus et al. (2015) reported that AB and BB genotypes were linked to milk lactose and somatic cell score in Karagouniko sheep. Similarly, Fadhil and Dakheel (2022) noted higher milk lactose percentages in AB and BB genotypes compared to the AA genotype in Awassi sheep.

CONCLUSION

In summary, this study identified only AA and AB genotypes in the *PAEP* gene exon II region in cross-bred Hamdani sheep, with a higher A allele frequency. The AA genotype exhibited an association with milk fat percentage, while no such association was observed with other milk composition traits. Consequently, *PAEP* genotypes hold promise for enhancing milk composition in cross-bred Hamdani sheep through breeding programs.

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Ethical statement or informed consent: The study was approved by Siirt University Animal Experiments Local Ethics Committee (Approval no: 2023-01-10).

Author contributions: AOT conceived the idea, arranged necessary funding, carried out experimental work and prepared original draft of the manuscript. EG carried out software and preparation of original draft. DK also conceived the idea, helped in experimental work, preparation of original draft. SÜ carried out experimental work. All authors reviewed and approved final version of the manuscript.

Availability of data and materials: The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

Anton, Z., Istvan, K., Attila, M., Sandor, F., Andras, L. (1998). Genetic polymorphisms of milk proteins in Hungarian dairy sheep breeds and crosses, Sheep and Goat Production in Central and Eastern European Countries, Hungary. *REU Technical Series, 50,* 224-226.

Baranyi, M., Kerekes, A., Hiripi, L., & Bősze, Z. (2010). Preliminary data on beta-lactoglobulin genetic polymorphisms in Hungarian Awassi and Racka sheep. *Scientific Papers Animal Science and Biotechnologies*, *43*, 1-4.

Barbano, D. M., Clark, J. L., Dunham, C. E., & Flemin, R. J. (1990). Kjeldahl method for determination of total nitrogen content of milk: collaborative study. *Journal of the Association of Official Analytical Chemists*, *73*(6), 849-859. https://doi.org/10.1093/jaoac/73.6.849

Bayraktar, M., & Shoshin, O. (2021). Determination effects of slc27a3 and β-lactoglobulin gene polymorphisms on the milk composition in Hamdani sheep. *Applied Ecology and Environmental Research*, *19*(4), 3293-3302. http://dx.doi.org/10.15666/aeer/1904_32933302

Corral, J. M., Padilla, J. A., & Izquierdo, M. (2010). Associations between milk protein genetic polymorphisms and milk production traits in Merino sheep breed. *Livestock Science*, *129*(1-3), 73-79. https://doi.org/10.1016/j.livsci.2010.01.007

Dario, C., Carnicella, D., Dario, M., & Bufano, G. (2008). Genetic polymorphism of β-lactoglobulin gene and effect on milk composition in Leccese sheep. *Small Ruminant Research*, *74*(1-3), 270-273. https://doi.org/10.1016/j.smallrumres.2007.06.007

Đokić, M., Marković, B., Marković, M., Mirecki, S., Veljić, M., & Perić, L. (2019). Relationship between β-Lactoglobulin gene polymorphism and milk traits and milk composition of Sora sheep breed. In *International Symposium on Animal Science (ISAS): Herceg Novi, Montenegro*,100-105. https://doi:10.17707/AgricultForest.66.3.02

Eignatev, G. (1998). Genetic polymorphisms of milk proteins in Russian sheep breeds and crosses. [Doctoral Dissertation, Moscow University, Russia].

Erhardt, G. (1989). Evidence for a third allele at the β-lactoglobulin (BLG) locus of sheep milk and its occurrence in different breeds. *Animal Genetics*, *20*, 197-204. https://doi.org/10.1111/j.1365-2052.1989.tb00857.x

Fadhil, I. A., & Dakheel, M. H. (2022). Impact of BLG gene polymorphism on some ovine production traits reared in middle part of Iraq. *Caspian Journal of Environmental Sciences*, *20*(5), 1003-1009. https://doi.org/10.22124/CJES.2022.6056

Falconer, DS., & Mackay, T.E.C. (1996). *Introduction to Quantitative Genetics*, (4th. ed.) Longman, Essex.

Feligini, M., Parma, P., Aleandri, R., Greppi, G. F., & Enne, G. (1998). PCR-RFLP test for direct determination of beta-lactoglobulin genotype in sheep. *Animal Genetics*, *29*(6), 473-474. https://doi.org/10.1046/j.1365-2052.1998.296352.x

Giambra, I.J., Jäger, S., & Erhardt, G. (2010). Isoelectric focusing reveals additional casein variants in German sheep breeds. *Small Ruminant Research*, *90*(1-3), 11-17. https://doi.org/10.1016/j.smallrumres.2009.12.025

Kawecka, A., & Radko, A. (2011). Genetic polymorphism of β-lactoglobulin in sheep raised for milk production. *Journal of Applied Animal Research*, *39*(1), 68-71. https://doi.org/10.1080/09712119.2011.565223

Kleyn, D.H., Lynch, J. M., Barbano, D. M., Bloom, M. J., Mitchell, M. W., Cooper, L. S., Cusak, E., Fick, M., Hanks, T., Hesen, M. K., Johnson, J., Kleyn, D. H., Mercer, F., Monahan, D., Peat, B., & Petit, M. (2001). Determination of fat in raw and processed milks by the Gerber method: Collaborative study. *Journal of AOAC International*, *84*(5), 1499-1508. https://doi.org/10.1093/jaoac/84.5.1499

Koca, D., Turgut, A. O., Çetin, N., Üner, S., Gülendağ, E., & Karagülle, B. (2023). Chemical composition and physical properties of milk in Norduz sheep. *Van Veterinary Journal*, *34*(3), 271-274. https://doi.org/10.36483/vanvetj.1353378

Kolde, H.J., & Braunitzer, G. (1983). The primary structure of ovine βlactoglobulin. *Milckwissenschaft, 38*, 70-72.

Komprej, A., Gorjanc, G., Kompan, D., & Kovač, M. (2012). Lactation curves for milk yield, fat, and protein content in Slovenian dairy sheep. *Czech Journal of Animal Science*, *57*(5), 231-239. https://doi:10.17221/5921-CJAS

Kusza, S., Ilie, D. E., Sauer, M., Nagy, K., Atanasiu, T. S., & Gavojdian, D. (2018). Study of LGB gene polymorphisms of small ruminants reared in Eastern Europe. *Czech Journal of Animal Science*, *63*(4), 152-159. https://doi:10.17221/93/2017-CJAS

Mele, M., Conte, G., Serra, A., Buccioni, A., & Secchiari, P. (2007). Relationship between beta-lactoglobulin polymorphism and milk fatty acid composition in milk of Massese dairy ewes. *Small Ruminant Research*, *73*(1-3), 37-44. https://doi.org/10.1016/j.smallrumres.2006.10.021

Michalcova, A., & Krupova, Z. (2009). Influence of β-lactoglobulin C genotypes on composition of milk and milk production traits of the Slovak ovine breeds. *Acta fytotechnica et zootechnica–Mimoriadne číslo Nitra, Slovaca Universitas Agriculturae Nitriae*, *12*, 438-446.

Mohammadi, A., Nassiry, M.R., Elyasi, G., & Shodja, J. (2006). Genetic polymorphism of β-lactoglobulin in certain Iranian and Russian sheep breeds. *Iranian Journal of Biotechnology*, *4*, 265-268

Othman, O. E., El Fiky, S. A., Hassan, N. A., Mahfouz, E. R., & Balabel, E. A. (2012). Genetic polymorphism of whey protein genes *β*-LG and *α*-LA in three Egyptian sheep breeds. *Journal of Applied Biological Sciences*, *6*(3), 25-30.

Özmen, Ö., & Kul, S. (2016). Investigating the genetic polymorphism in the exon 2 region of ovine beta-lactoglobulin gene and its association with some milk traits. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, *63*(3), 323-328.

Russel, A.J.F., Doney, J.M., & Gunn, R.G. (1969). Subjective assessment of body fat in live sheep. *Journal of Agricultural Science (Cambridge) 72*, 451–454. https://doi.org/10.1017/S0021859600024874

Rustempašić, A., Dokso, A., Zečević, E., Hodžić, A., Hrković-Porobija, A., Sarić, Z., Miskoska–Milevska, E., Popovski, Z., & Brka, M. (2018). Polymorphism of β-lactoglobulin in Pramenka sheep breed in Bosnia and Herzegovina. *The Journal of Animal & Plant Sciences*, *28*(1), 337-340.

Selvaggi, M., Laudadio, V., Dario, C., & Tufarelli, V. (2014). Investigating the genetic polymorphism of sheep milk proteins: A useful tool for dairy production. *Journal of the Science of Food and Agriculture*, *94*(15), 3090-3099. https://doi.org/10.1002/jsfa.6750

Staiger, E. A., Thonney, M. L., Buchanan, J. W., Rogers, E. R., Oltenacu, P. A., & Mateescu, R. G. (2010). Effect of prolactin, β-lactoglobulin, and κ-casein genotype on milk yield in East Friesian sheep. *Journal of Dairy Science*, *93*(4), 1736-1742. https://doi.org/10.3168/jds.2009-2630

Stambekov, S., Shapiro, Y. O., & Mandrusova, E.E. (1997). Milk protein polymorphism in Soviet Merino and Latvian Dark headed ewes and its connection with economically valuable characteristics. *Journal of Dairy Science*, *39*, 6873.

Sumantri, C., Nurhayati, D., Farajallah, A., & Anggraeni, A. (2008). Association between polymorphism of β-lactoglobulin gene on milk yield and quality in local sheep at jonggol animal science teaching and research unit (Jastru). *In Annales Bogorienses*, *12*(1), 17-24.

Triantaphyllopoulos, K. A., Koutsouli, P., Kandris, A., Papachristou, D., Markopoulou, K. E., Mataragka, A., Massouras, M., & Bizelis, I. (2017). Effect of β-lactoglobulin gene polymorphism, lactation stage and breed on milk traits in Chios and Karagouniko sheep breeds. *Annals of Animal Science*, *17(2)*, 371-384. https://doi.org/10.1515/aoas-2016-0058

Wendorff, W. L., & Haenlein, G. F. W. (2017). Sheep milk-composition and nutrition. In. Young, W. P., George, F. W. H., & William, L. (Eds), *Handbook of Milk of Non-Bovine Mammals* (pp. 210-221). JohnWiley & Sons Ltd.

Yousefi, S., Azari, M. A., Zerehdaran, S., Samiee, R., & Khataminejhad, R. (2013). Effect of β-lactoglobulin and κ-casein genes polymorphism on milk composition in indigenous Zel sheep. *Archives Animal Breeding*, *56*(1), 216-224. https://doi.org/10.7482/0003-9438-56-021

TUIK., (2022, 5 May). *Çiğ Süt Üretim İstatistikleri 2022*. https://data.tuik.gov.tr/Bulten/Index?p=%C3%87i%C4%9F-S%C3%BCt-%C3%9Cretim%C4%B0statistikleri-2022-49699&dil=1

Turgut, A. O., Gulendağ, E, Koca, D., & Üner, S. (2023). Milk composition traits of Hamdani cross-bred sheep raised under extensive management. *ISPEC Journal of Agricultural Science*, *7*(2), 271-279. https://doi.org/10.5281/zenodo.8020354