



Tunisian Autochthonous Goats Display a Prevalence of Alpha S1 Casein Genetic Variants Associated with High Protein Content in Milk

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ABSTRACT

Goat milk possesses exceptional nutritional qualities and wide-ranging technological applications, which are influenced not only by the conditions of animal breeding but also by the genetic variability in casein genes. The present work assesses the variability of *Alpha S1 (CSN1S1)* casein gene in a goat population raised in Southeast Tunisia, which has consistently proven extraordinary adaptability to the challenging conditions including water scarcity and limited access to feed resources. A total of 45 unrelated goats belonging to the native goat population of Tunisia were analyzed for the cited caseins using PCR-based methods (AS-PCR and PCR-RFLP). The CSN1S1 locus, screening for seven alleles (A, B, C, E, F, O1, and N) revealed six variants, resulting in 13 genotypes. Among the observed alleles, the strong alleles A, B, and C were the most prevalent with an allelic frequency of more than 70%. Interestingly, the null allele O1 was detected in only one animal. Moreover, the prevalence of the strong alleles of the CSN1S1 gene, is recognized for its association with higher milk protein content. This finding suggests that these goats carry genetic traits that contribute to the production of protein-rich milk. It also highlights their adaptation to harsh conditions, demonstrating their ability to support the well-being and survival of their young. This adaptive trait enables them to mitigate the impact of water scarcity and limited feed resources on the growth and development of their offspring. Such information remains essential for refining breeding strategies and improving milk production outcomes.

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Authors' Contribution

SK performed the laboratory analysis, drafted the manuscript, analyzed the data, and reviewed the manuscript. MH, TK and MBH provided resources and samples for the study and reviewed the manuscript. MHY designed the laboratory experiments, supervised the research, and reviewed the manuscript. All authors have read and approved the final manuscript.

Key words

Tunisian goat, CSN1S1 gene, Genetic polymorphism of milk casein, Arid climate, Adaptation, Alpha S1 casein, Strong alleles

INTRODUCTION

The characterization and study of goats, as well as the quality of their products in their original environments, are of great importance. The composition of goat milk, particularly the presence of casein proteins, plays a crucial role in its nutritional quality and technological properties (Nayik *et al.*, 2022; Rahmatalla *et al.*, 2022; Campos *et al.*, 2022; Al-Kaisy *et al.*, 2023). Caprine milk contains four casein proteins: alpha-S1-casein, beta-casein, alpha-S2-casein, and kappa-casein, encoded by the CSN1S1, CSN2, CSN1S2, and CSN3 genes, respectively. Genetic

polymorphisms of milk casein in goats have been extensively analyzed in several countries, and all four genes exhibit various genetic variants. The effect of such polymorphism has been proved for several breeds (Dagnachew *et al.*, 2011; Vacca *et al.*, 2014; Zhang *et al.* 2019; Rahmatalla *et al.* 2022; Dettori *et al.*, 2023). In addition, Meena *et al.* (2021) and Rahmatalla *et al.* (2022) reported that these genetic variants affect the features of milk processing, human nutrition and health, and environmental adaption. These authors also noted that particular breeding objectives are reflected in the high prevalence of particular alleles of caseins in various goat breeds.

The genetic polymorphism within the CSN1S1 in goat milk affects the amount of protein and fat produced (Ollier *et al.*, 2008; Ballabio *et al.*, 2011; Singh *et al.*, 2018; Zhang *et al.*, 2019; Tumino *et al.*, 2023). So far, 22 protein variants have been reported in goats for alpha-S1-casein (Cosenza *et al.*, 2003; Rahmatalla *et al.*, 2022). These variations are associated with the content of *as1*-Cn in milk, thus they are categorized into four groups: 'strong' (approximately 3.5 to 4.2 g/l), 'intermediate' (~1.1 to 1.6

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g/l), 'weak' (~0.45 to 0.6 g/l) and 'null' (0.0 g/l) alleles. Within the strong alleles 13 (A, A2, A3, A', B', B1, B2, B3, B4, C, H, L, and M) have been identified. Both for the intermediate and weak alleles, only three have been characterized, E, I, D1 and D, F, G, respectively. Only 01, 02, and N alleles are associated the absence of this casein in milk. Recently, [Zhang *et al.* \(2019\)](#) reported that alpha-S1-casein polymorphism has a significant impact on milk composition (proteins and lipids), thereby affecting the quality and technological properties of goat milk ([Zhang *et al.*, 2019](#)).

The estimated size of Tunisia's caprine herd was 741,560 female units in 2017 ([Onagri, 2024](#)). This herd is mainly composed of Tunisian native goat population, known as "Arbi" goats. These goats are distributed across various regions of Tunisia, with a particular concentration in the southern areas ([Nafti *et al.*, 2016](#)).

In the southern region, native goats ([Fig. 1](#)) represent an interesting animal genetic resource, and show exceptional adaptation to the arid and harsh conditions specific to this region. With their small size, long coats, and ability to thrive in extreme environments, these goats have evolved to survive with limited water (cumulated precipitation is less than 50 mm) and temperatures of up to 47°C during hot, dry seasons ([Ammar *et al.*, 2017](#); [Onagri, 2024](#)).



Fig. 1. Photo of Tunisian native goats in the desert of southern Tunisia.

In these areas, kidding is concentrated in autumn-winter ([Ammar *et al.*, 2011](#)). Consequently, kids must be able to thrive and grow during the first dry season, only few months after weaning.

These goats are highly efficient in valorizing the scarce resources and agricultural by-products, thereby contributing to sustainable agricultural inputs ([Chniter *et al.*, 2023](#)). Furthermore, the native goat husbandry

plays a capital role by significantly contributing to farmers' incomes ([Gaddour and Najari, 2009](#)). In last decades, goat milk was rarely sold, it was consumed by the household. However, an increasing number of farmers are getting interested in selling their goats' milk due to its profitable price (equivalent to 0.70\$/liter). In this regard, investigating the genetic polymorphism of milk protein is essential for optimizing breeding strategies and improving milk production outcomes. In fact, genetic polymorphisms of milk casein in goats have been analyzed in several countries. However, little of information ([Ouni *et al.*, 2007](#); [Jemmali *et al.*, 2012](#)) is available on the native goat population of Tunisia. The present work aims to investigate the genetic variations of the *CSN1S1* in the native goat population raised in the southernmost of Tunisia.

MATERIALS AND METHODS

DNA samples

A total of 45 blood samples were collected from unrelated native goats in southern of Tunisia. The collection of these samples for this study was carried out during the routine animal sanitary controls by an authorized veterinarian. Genomic DNA was extracting using the standard phenol-chloroform method ([Sambrook *et al.*, 1989](#)).

Genotyping at the *CSN1S1* locus

Alleles at the *CSN1S1* gene were detected using PCR based methods: The AS-PCR method for the alleles *CSN1S1* E and ([Cosenza *et al.*, 2003](#); [Pérez *et al.*, 1994](#)). RFLP-PCR was optimised to detect *CSN1S1* C using *HphI* ([Cosenza *et al.*, 2008](#)) and to discriminate A, B, N and F variants using *XmnI* ([Ramunno *et al.*, 2000](#)). Primer sequences and the thermal amplification conditions are reported in [Table I](#).

The PCR reaction contained 125 ng of genomic DNA, 0.4 µM of each primer, 0.2 mM dNTPs, 2 mM MgCl₂, and 0.5 unit of Taq polymerase (Fermentas) in a total volume of 25 µL. The amplification protocol consisted of an initial denaturation step at 94 °C for 1 min 30 s. The next 35 cycles were performed using the following conditions: 94 °C for 45 s, T_m of each primer for 1 min, 72 °C for 1min and the finale extension was carried out at 72 °C for 5 min.

The total volume of reaction digestion was 20 µL. It contained 10X Buffer, 10 µl PCR product, 8 unit of restriction enzyme, and ddH₂O. The incubations were carried out at 37°C for an overnight. Digested products were analyzed using electrophoresis on 2% agarose gel in 1X TAE buffer stained with ethidium bromide. A Bio-Print 1000 was used to visualize band patterns. Restriction enzymes and corresponding positions are reported in [Table II](#).

Table I. Oligonucleotide primers and its T_m for PCR-RFLP and AS-PCR assays. X: include A*, B* group alleles, N and F alleles.

Allele	Primer	Sequence (5'-3')	T _m (°C)	PCR Product (pb)	References
C	CSN1S1_C-F	AACAGCACTGTTAAATGTATAAT	60	194	Cosenza <i>et al.</i> , 2008
	CSN1S1_C-R	TCATCAGTTAAGCTACACAA			
E	CSN1S1_E-F	TCCATGCTTTACATGTCTTTTC	60	172	Designed in this study
	CSN1S1_E-R	AACAAGCTCTTAGGACAATTC			
« X »	CSN1S1_X-F	TTCTAAAAGTCTCAGAGGCAG	60	212-224	Ramunno <i>et al.</i> , 2000
	CSN1S1_X-R	GGGTTGATAGCCTTGATGT			
O1	CSN1S1_O1-F	CCCCAGCTGGTAATGTTTTA	60	249-281	Sztankóová <i>et al.</i> , 2006
	CSN1S1_O1-R1	GGTCCATCAATTCCCTGTGT			
	CSN1S1_O1-R2	TGTATGGATCCCTGATTCCTTC			

Table II. Positions and restriction enzymes for PCR-RFLP.

Enzyme	Restriction site	Incubation temperature (°C)	Incubation period
HphI (Fermentas)	5'...GGTGA(N) ₈ ↓...3' 3'...CCACT(N) ₇ ↑...5'	37	overnight
XmnI (PdmI) (Fermentas)	5'...GAANN↓NNTTC...3' 3'...CTTNN↑NNAAG...5'	37	overnight

Statistical analysis

The allele and genotype frequencies were estimated by direct counting. Moreover, Hardy-Weinberg test was conducted using Genepop V4.7.0 (Rousset, 2008). It was evaluated for each genetic locus in the studied population using a locus-by-locus test method. This involved running the Markov chain with 1000000 steps and 100000 dememorization steps. Additionally, the chi-square (χ^2) test was employed to detect any deviations from the Hardy-Weinberg equilibrium within the population, with a significance level set at $p \leq 0.05$.

$$\chi^2 = \sum ((O - E)^2 / E)$$

In the equation, O is the frequencies of observed genotypes, E is the frequencies of the expected genotypes and $\sum$ is “the sum of”.

RESULTS

In the present work, we investigated the genetic polymorphism of CSN1S1 locus across goats raised in southernmost arid region of Tunisia and belonging to the native goat population. A total of 48 samples have been genotyping the alleles C, E, O1 as well as those of type A (A* = A, G, H, I, and O2) and B (B* = B1, B2, B3, B4, and L) of CSN1S1 gene. The P-value for the Hardy-Weinberg test indicated that the analyzed population was in Hardy-Weinberg equilibrium for the two loci.

The AS-PCR method allowed the identification of animals with the CSN1S1 E allele. The presence of this variant is shown on an amplified 172-bp fragment belonging to exon 19. Whereas, the amplified fragment characterizing the CSN1S1 O1 allele was 249 bp long and spans a part of the 12th exon and a part of the 12th intron.

Typing 45 animal DNA samples confirmed the absence of the CSN1S1 E allele in this population. Besides, the null allele O1 was detected in only one animal in the heterozygous form (Figure 2, Table III).

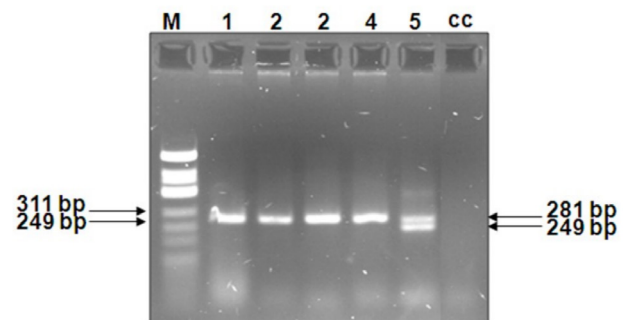


Fig. 2. DNA electrophoretic patterns of the amplified fragments characterizing the goat CSN1S1A and CSN1S1O1 alleles of goat CSN1S1 casein gene obtained by using AS-PCR. M: Marker (Φ x174 Hinf I); 1, 2, 3, 4: AA; 5: AO1 and cc: contamination control.

Table III. Frequencies of different alleles of alpha S1 casein (*CSN1S1*) found in Tunisian native goat population.

Alleles	Frequency
A	0.267
B	0.489
C	0.033
E	0.000
F	0.078
N	0.122
O1	0.011

The amplified fragments corresponding to the *CSN1S1* C and *CSN1S1* non-C alleles are distinguished by the presence and absence, respectively, of the *HphI* restriction site. Among the 45 samples analyzed, three displayed heterozygosity (194/111+83 bp), while the remaining samples exhibited homozygosity for the 111+83 bp fragment.

The use of PCR-RFLP with the *XmnI* restriction enzyme revealed the following genotypes: A*A*, A*B*, A*F, A*N, B*B*, B*F, B*N, FN, NN. Given the rarity of variants G, H, I, L, and O2, specific protocols were employed to genotype alleles C, E, and O1. Consequently, group A* is considered equivalent to the A allele, and group B* to the B allele for the subsequent analysis.

Table IV. Frequencies of different genotypes of alpha S1 casein (*CSN1S1*) found in Tunisian native goat population.

Genotypes	N ^a	Frequency
AA	4	0.088
AB	8	0.177
AC	1	0.022
AF	4	0.088
AN	3	0.066
BB	15	0.333
BC	1	0.022
BF	1	0.022
BN	3	0.066
BO1	1	0.022
CF	1	0.022
FN	1	0.022
NN	2	0.044

^a, number of individuals.

Table IV summarizes the genotyping results for casein α S1 in the studied population, identifying six alleles (A, B, C, F, N, and O1) across thirteen distinct genotypes: AA, AB, AC, AF, NA, BB, BC, BN, BO1, CF, FN and NN.

DISCUSSION

Goat milk contains numerous proteins, with the six main ones being the caseins (α S1, β , α S2, and κ) and the two major whey proteins, β -lactoglobulin and α -lactalbumin. The four caseins, which precipitate at pH 4.6 and 20 °C, represent approximately 80% of the total milk proteins. They serve as the primary protein source in cheese production. These caseins are present in milk as sub-micelles, which assemble into micelles, forming very fine and insoluble spherical particles. The kappa-casein plays a crucial role in ensuring the formation and stability of the micelles.

Caseins are encoded by a group of four loci on a 250 Kb region, arranged in the following order on chromosome 6: α S1, β , α S2, and κ (Grosclaude et Martin, 1997; Hayes et al., 1993; Popescu et al., 1996). The α S1 gene exhibit varying level of polymorphism among the three species of domestic ruminants (cattle, sheep, goats). In sheep, nine alleles, including A, B, C, D, E, F, H, G, and I, have been identified (Giambra et al., 2010a, b), while in cattle populations, nine variants (A, B, C, D, E, F, H, G, and I) have been described (Kishore et al., 2013; Tolénkhomba et al., 2021). In the caprine, the highest level of polymorphism is observed, with the presence of at least 22 alleles (Cosenza et al., 2003; Rahmatalla et al., 2022). Among these alleles, at least 15 alleles have been associated with four different efficiencies of protein production (Ramunno et al., 2004): strong alleles (A, B, C, H, L, M), intermediate alleles (E and I), weak alleles (F and G), and null alleles (O1, O2, N) (Ramunno et al., 2004; Rando et al., 2000; Johansson et al., 2023). This highlights how variations α S1-casein encoded by the *CSN1S1* gene, are closely associated with milk protein quality and quantity in goats (Dettori et al., 2023).

Compared with cows, Tunisian native goat showed an impressive ability to survive and product in harsh environment in water-limited regions especially in the southernmost arid and desertic regions of the country. Within these water restriction conditions and since milk is considered an alternative for animal protein, it seems interesting to investigate notably the genetic variation of milk caseins.

The strong alleles A, B, and C exhibit high frequencies in this population (approximately 0.79), consistent with findings from various studies by Ouafi et al. (2002), Ouni et al. (2007), and Moili et al. (2007) that highlight the

predominance of strong alleles in goat populations in Morocco and Tunisia. Remarkably, the B and A alleles are more prevalent at 0.489 and 0.267, respectively, while the contribution of the C allele is notably lower at 0.033. These findings could be explained by the exceptional adaptability of goats to challenging conditions of water scarcity and limited access to feed resources. By providing their offspring with protein-rich milk, goats facilitate rapid growth in their kids before the onset of the estival season, characterized by high temperatures and scarce feed resources. This adaptive strategy plays a crucial role in ensuring the survival and well-being of the goat population during the most challenging period of the year.

The frequency of the F allele is relatively modest at 0.078, aligning with results reported by Ouni *et al.* (2007) and studies on Spanish, African, and Black-Rahall Moroccan goat breeds (Ouafi *et al.*, 2002; Jordana *et al.*, 1996).

Among the 45 individuals examined, the null allele O1 was identified in only one animal in a heterozygous state. This low frequency (0.011) is akin to the findings of Ouni *et al.* (2007) but notably lower than those stated in European goat breeds (Ouafi *et al.*, 2002; Grosclaude *et al.*, 1987; Caroli *et al.*, 2007). When considering the N allele, the combined frequency of null alleles (N + O1) amounts to 0.133 in the population under study. This frequency surpasses those observed in Moroccan populations as reported by Ouafi *et al.* (2002).

CONCLUSIONS

Our study delves into the genetic diversity of casein genes in the goat population of Southeast Tunisia, specifically focusing on the Alpha S1 (*CSN1S1*) gene. By analyzing 45 unrelated goats from the native Tunisian goat population using PCR-based methods, the research uncovered six variants at the *CSN1S1* locus, leading to 13 genotypes. The results not only highlighted the genetic diversity present in Tunisian native goats but also provided valuable insights into the protein composition of their milk. This finding shed light, namely, on the adaptation of this goat to arid environment.

DECLARATIONS

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IRB approval

None

Ethical statement

All blood samples used in the present work were taken during routine animal sanitary controls by an authorized veterinarian.

Generative AI or AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflicts of interest

The authors declare no conflict of interest.

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