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Research Article

Genetic Variants of Kappa Casein in Tunisian Native Goats

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ABSTRACT

Introduction: The nutritional qualities of goat milk are affected by both environmental factors and genetic variations within casein genes. This study aims to assess the genetic polymorphism of the *Kappa Casein* (*CSN3*) gene in a goat population from Southeast Tunisia. This population is known for its exceptional resilience to harsh conditions, including limited feed and water scarcity.
Materials and methods: The PCR-RFLP was used to analyze the genomic DNA of 48 blood samples belonging to unrelated individuals from the Tunisian goat population for the *CSN3* casein gene variability, at positions 166 and 448 bp.
Results: The analysis revealed a high prevalence of the GG genotype at position 166 and the TT genotype at position 448. Interestingly, the frequency of alleles from group I (A, B, E, F, H, J, and K) in the studied goat population of the present work is quite high; the alleles belonging to this group were known as associated with higher milk protein content. These findings suggest that these goats possess genetic traits that may improve milk protein production, which is crucial for ensuring the survival and well-being of their offspring.
Conclusion: The prevalence of alleles within the *CSN3 gene*, which is associated with high milk protein content, is a notable finding in this study. These genetic characteristics help mitigate the negative impacts of restricted feed and water on the growth and development of the offspring. The present study displays one of several adaptative features of this goat population which may highlight also the importance of these traits for sustainable goat breeding and milk quality improvement.

1. Introduction

Climate change underlines the need to understand goats and the quality of their products in their native environments. Goat milk composition, particularly its casein proteins, plays a major role in both its nutritional benefits and its use in various technological applications[1-4]. This milk contains four main casein proteins including beta-casein, alpha-S1-casein, alpha-S2-casein, and kappa-casein, encoded by the *CSN2*, *CSN1S1*, *CSN1S2*, and *CSN3* genes, respectively. Research across different countries has shown significant genetic differences in these casein genes, which affect milk traits in various breeds[5-8]. Rahmatalla et al.[2] and Meena et al.[9] also highlighted that these genetic variations influence milk's processing qualities, nutritional value, health benefits, and ability to adapt to the harsh environment.

Like other caseins, κ-casein significantly impacts the nutritional quality of milk[2]. The *CSN3* gene in goats is highly diverse, with 24 non-synonymous variants documented[2]. Variants such as CSN3*D, CSN3*E, CSN3*K, and CSN3*M, have been found to influence protein levels in milk, particularly casein, according to studies by Chiatti et al. [10,11] and Caravaca et al.[12]. Exploring the genetic diversity of κ-casein is crucial for improving milk quality, refining breeding programs, and boosting productivity in dairy goat populations.

In Tunisia, the goat population was estimated at 741560 females in 2017[13], with the majority being native goats. These goats are mainly found in southern regions of the country[14]. Native goats in this area are an essential genetic resource, known for their ability to adapt to harsh arid conditions. They can thrive with minimal water—less than 50 mm of annual rainfall and tolerate high temperatures of up to 47°C during

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dry seasons[15,16].

These goats make efficient use of limited resources and agricultural byproducts, contributing to more sustainable farming practices[17]. Their role in supporting farmers' incomes is also significant[18]. While goat milk has traditionally been consumed at the household level, more farmers are now selling it commercially due to its favorable market price (around $0.70 per liter). Studying genetic polymorphisms in milk proteins is therefore essential for improving breeding strategies, increasing production, and enhancing the value of native goat populations. This study aimed to investigate the genetic variation within the *CSN3* gene.

2. Materials and Methods

2.1. Ethical approval

This study was conducted according to the guidelines of the Livestock and Wildlife Laboratory, Institut des Régions Arides, Tunisia.

2.2. DNA Samples

An average of 3 mL blood samples was collected using a 5 mL EDTA tube from 48 unrelated native goats in southern Tunisia during routine animal sanitary controls conducted by an authorized veterinarian. Genomic DNA was extracted using the standard phenol-chloroform method[19].

2.3. Genotyping at the CSN3 Locus

RFLP-PCR using *BseNI* and *Alw44*I was utilized to discriminate the CSN3 alleles at positions 166 and 448 respectively. A total of 125 ng of genomic DNA was amplified in a volume of 25 µL containing specific primers[24] (KB1F: 5'-TGTGCTGAGTAGGTATCCTAGTTATGG-3' and KB2R: 5'-GCGTTGTCCTCTTTGATGTCTCCTTAG-3'), dNTPs, MgCl2, and Taq polymerase.

The PCR reaction was prepared in a 25 µL total volume, including 125 ng of genomic DNA, 0.4 µM of each primer, 0.2 mM dNTPs, 2 mM MgCl2, and 0.5 units of Taq polymerase (Fermentas).

The amplification started with an initial denaturation at 94 °C for 1 minute and 30 seconds. This was followed by 35 cycles with these steps including 94 °C for 45 seconds, the annealing temperature (Tm: 63°C) of the primers for 1 minute, and 72 °C for 1 minute. Finally, the reaction ended with 5 minutes of final extension at 72 °C.

The digestion reaction had a total volume of 25 µL. It included 10X buffer, 10 µL of PCR product, 8 units of restriction enzyme, and ddH2O.

Incubation is carried out at 37°C overnight (for *Alw44I*) and at 65°C for 6 hours (*BseNI*). The digested products were checked using electrophoresis on a 2% agarose gel with 1X TAE buffer and stained with ethidium bromide. The band patterns were visualized using Bio-Print 1000.

2.4. Statistical Analysis

The allele and genotype frequencies were calculated by direct counting. The *Hardy-Weinberg equilibrium* was tested for positions 166 and 448 in the *CSN3* gene using the Genepop software (version 4.7.0)[20].

3. Results and Discussion

Goat milk contains several proteins including four caseins (αS1, β, αS2, and κ) and two whey proteins (β-lactoglobulin and α-lactalbumin). Caseins make up around 80% of the total milk proteins and are important for cheese making. These proteins form micelles, which are tiny, insoluble spherical particles, with κ-casein helping to stabilize them[2,31].

The genes for caseins are located on a 250 Kb region of chromosome 6 in this order: αS1, β, αS2, and κ.[21-23]

3.1. Mutation at position 166

This mutation is located at position 166 of exon 4 (position 309 in cDNA). It involves a G/A transition leading to protein variability at position 65, where the valine residue (AGT) is substituted with the isoleucine residue (AAT).

This mutation results in the abolition of a *BseNI* endonuclease restriction site. Thus, the PCR-RFLP method was used to study this polymorphism. A fragment of 459 bp in exon 4 was amplified using the primer pair (KB1-KB2), followed by digestion with BseNI. Electrophoresis on a 2% agarose gel revealed the presence of three fragments of 354 bp, 54 bp, and 51 bp for the G allele (Valine), and only two fragments of 405 bp and 54 bp for the A allele (Isoleucine).

Figure 1 displays the two genotypes obtained including GG homozygote (valine) (354 + 54 + 51 bp) and GA heterozygote (valine-isoleucine) (354 + 405 bp). However, no AA homozygotes (valine) were observed among all 48 individuals analyzed. The GG genotype was detected in 42 individuals (87.5%), while the GA genotype was found in only 6 individuals (12.5%).

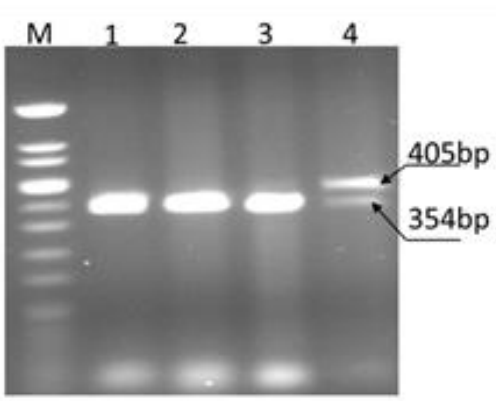

Figure 1. Electrophoretic profile on a 2% agarose gel of the fragments obtained after PCR-RFLP using the BseNI restriction enzyme for genotyping the samples at position 166 of the *CSN3* locus in goat. M: Molecular weight marker (Φx174 Hinf I), 1-3: GG homozygotes, 4: GA heterozygote. The 54 bp and 51 bp fragments are not visible on the gel.

3.2. Mutation at position 448

This polymorphism corresponds to a T>C base change at position 448 of exon 4 (591 in cDNA) of the kappa-casein gene. This alteration results in the substitution of the amino acid serine by proline at position 159 of the mature protein. Additionally, it creates a restriction site for the *Alw44I* enzyme, enabling its detection through the PCR-RFLP method. Digestion of the PCR product by this enzyme yields two fragments of 381 and 78 bp for the C allele, while it remains

complete (459 bp) for the T allele (Figure 2).

Figure 2 shows the three genotypes obtained including TT homozygote (serine) (459 bp), TC heterozygote (serine-proline) (459 + 381 bp), and CC homozygote (proline) (381 bp). Among the 48 individuals analyzed, 37 (77%) are homozygous T/T, 10 (21%) are heterozygous, and only one individual is homozygous C/C. The distribution of the T/T, C/C, and T/C genotypes among the individuals studied, as well as the allelic frequency distribution, are presented in Tables 1 and 2.

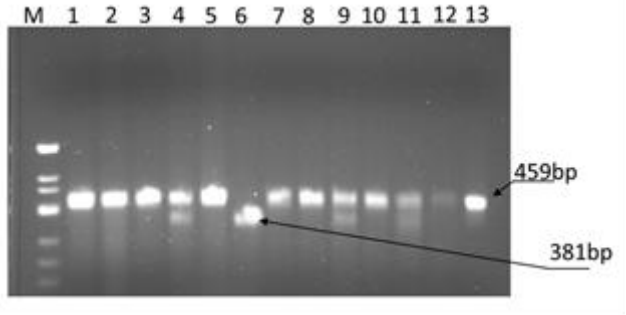

Figure 2. Electrophoretic profile on a 2% agarose gel of the fragments obtained after PCR-RFLP using the Alw44I restriction enzyme for genotyping the samples at position 448 of the *CSN3* locus in goat. M: Molecular weight marker (Φx174 Hinf I), Samples 4, 9 and 11: heterozygote CT, sample 6: homozygote CC and other samples are homozygotes TT. The fragment 78 is not visible on the gel.

Table 1. Frequencies of different genotypes of *Kappa casein* gene found in Tunisian native goat population. N: number of individuals.

Gene	Genotypes	Number	Frequency
CSN3 position166	GG	42	0.875
	GA	6	0.125
	AA	0	0.000
CSN3 position 448	TT	37	0.770
	TC	10	0.210
	CC	1	0.020

Table 2. Frequencies of different alleles of *Kappa casein* gene found in Tunisian native goat population.

Gene	Alleles	Frequency
CSN3 position166	G	0.975
	A	0.625
CSN3 position 448	T	0.875
	C	0.125

In this study, the genetic polymorphism of the *kappa casein* gene has been analyzed by genotyping mutations at positions 166 and 448 of exon 4 in 48 samples belonging to the native goat of Tunisia. Thus, a fragment of 59 bp of this exon has been amplified using PCR and analyzed using the *RFLP* method to determine the genotype of the polymorphic sites. The G/A mutation at position 166 causes a change in the amino acid sequence of the mature kappa casein protein, and a substitution of valine with an isoleucine at position 65 in the N-terminal region occurred. This mutation was first reported in the French Saanen breed[24], and its genotyping allowed to differentiate between variants A, B, E, F, H, J, K, and M, which have the nucleotide G (valine), and variants C, D, G, I, and L, which have adenosine (isoleucine).

Similarly, the T/C mutation at position 448 leads to a substitution of the serine residue with proline in the C-terminal region of the protein (caseinomacropeptide). Genotyping this mutation could distinguish between variants A, B, E, H, I, J, and K, which have the nucleotide T (serine), and variants C, D, F, G, L, and M, which have cytosine (proline).

However, due to the high number of mutations responsible for the different variants of kappa casein, genotyping positions 166 and 448 do not provide an exact determination of an animal genotype. Instead, it only indicates their belonging to a group of alleles. Our results suggest that the frequency of alleles C, D, F, G, I, L, and M (group II) is relatively low in this native goat. This limited presence of these alleles has also been observed in most studied goat breeds[6,25-27]. Furthermore, these populations generally have limited geographical distribution and small population sizes.

Interestingly, the frequency of alleles from group I (A, B, E, F, H, J, and K) in the studied goat population of the present work is quite high (Table 2). Considering that alleles A and B are the most prevalent in almost all analyzed goat breeds, and the fact that other alleles in this group are rare and typically present in specific breeds, the frequencies observed in the Tunisian goat population could be broadly considered as those of variants A and B. In Italian and East African goats, allele B occurs at frequencies of more than 70%, while allele A is also highly prevalent in breeds such as Ionica and Montefalcone from Italy and Ardi goats from Saudi Arabia[2]. Rare alleles like E and C are poorly represented globally. For example, allele C was found at low frequencies only in certain populations like Murciano-Granadina goats[2,30]

Regarding the statistical analysis, at position 166, the results showed that *Hardy-Weinberg equilibrium* was matched. In Saanen goats, populations were also in Hardy-Weinberg equilibrium for the CSN3 locus[32]. The negative *Fis* value (-0.0455) indicates a small surplus of heterozygotes, which suggests that this position locus is naturally segregating without signs of selection or inbreeding. Similar trends were observed in Simmental cattle where heterozygosity at the *CSN3* locus was slightly elevated but within equilibrium limits[32].

For position 448, the *P-value* (0.4834) also showed no significant deviation from *Hardy-Weinberg equilibrium*, but a slightly positive *Fis* value (0.0841) revealed a small deficiency of heterozygotes. This could be due to mild inbreeding, population structure, or minor selection. This result could be explained by the genetic diversity and dynamics within the Southeast Tunisian goat population.

As for kappa casein, several studies have been conducted on the effect of the genotype on the milk composition and characteristics. Darwish et al.[24] reported an association between protein, fat, and lactose content in Egyptian goats with AG genotypes in the *CSN3* gene. Susilorini et al.[29] demonstrated an association between the composition and yield of milk produced by native Indonesian goats and its *CSN3* gene polymorphism. Dettori et al.[8] analyzed the variation of the casein gene and its association with several milk contents in the Italian Sarda goats. Their results showed a highly significant association with proteins; however, a weak association was found with lipid content, total solids, and milk energy. Association analysis conducted by Catota-Gómez et al.[30] also indicated that *CSN3* was significantly associated only with protein percentage in the Saanen goat breed of Mexico. In fact, the AC, BB, and CC genotypes might contribute to

favorable performance in protein percentage. Furthermore, the *CSN3* locus has been identified as a significant factor affecting the levels of total casein and protein in milk. Specifically, the AB and BB genotypes of the *CSN3* locus have been found to be associated with higher rates of total casein and protein content compared to the AA genotype in Murciano-Granadina goats, as shown by Caravaca et al.[12].

The result can provide insight into the high protein content of the milk produced by the studied population and shed light on the adaptive mechanisms adopted by these goats to ensure the survival and well-being of their kids, enabling them to thrive in harsh environments of water scarcity and limited access to feed resources, which could affect their growth.

4. Conclusion

In order to better understand the genetic diversity of the *Kappa casein* (*CSN3*) gene in goats from Southeast Tunisia, the authors of the current study used PCR-RFLP methods to analyze 48 unrelated goats and discovered that the most common genotypes were the GG genotype at position 166 and the TT genotype at position 448. These results demonstrate the rich genetic diversity of native Tunisian goats and provide important information about the composition of milk protein in these animals. Additionally, this study clarifies how this genetic variation may help these goats survive in harsh, arid environments.

Declarations

Competing interests

The authors declare that they have no competing interests.

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Ethical considerations

All authors have reviewed the manuscripts for ethical concerns, such as plagiarism, consent to publish, misconduct, data fabrication and falsification, double publishing and submission, and redundancy.

Authors' contributions

Samia Kdidi carried out the statistical analysis and wrote and reviewed the paper. Asma Majdoub carried out the laboratory analysis. Mohamed Habib Yahyaoui conceptualized and designed the study, carried out the laboratory analysis, supervised, and provided resources. All authors reviewed and confirmed the final manuscript.

Availability of data and materials

The data used in this study are available on request from the corresponding author.

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