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Polymorphism of Beta-Lactoglobulin and Kappa Casein Genes in Tunisian Native Goats

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Abstract: This study investigated genetic polymorphisms in the local goat populations of Tunisia. The polymorphisms of the β-lactoglobulin (β-LG) and kappa casein (CNS3) genes of two goat subpopulations in southern Tunisia (Jerid and Nefzawa regions), known as Arbi and Serti, were assessed at the DNA level. The DNA of 151 individual goats for β-LG and 171 individuals for CNS3 were extracted and then genotyped for β-LG using restriction fragment length polymorphism; SmaI restriction enzyme technique; and HaeIII restriction enzyme technique for CNS3. CNS3 gene digestion revealed two fragments of different sizes, 416 bp and 229 bp, indicating the presence of the AA and AB genotypes of kappa casein in the population. The AB genotype (645/416/229 bp) was also detected, while the BB genotype was completely absent in the oasis goat population. The results of the β-LG gene revealed three different genotypes, each characterized by a different number and size of fragments. The homozygous form CC presented three fragments of different sizes, 472, 181 and 50 bp, while the other form TT presented only two fragments, 472 and 231 bp. Finally, the heterozygous form CT controlled four fragments of different sizes, 472, 231, 181 and 50 bp. Allele A of kappa-casein occurred at a higher frequency than allele B in both the Arbi and Serti subpopulations and the overall population. The β-lactoglobulin C allele was more common than the T allele in the Arbi and Serti subpopulations and throughout the population. The genotypic frequencies of homozygotes were found to be higher than those of heterozygotes in the two subgroups and within the total population. At the CNS3 locus, the total population and the two subpopulations (Arbi and Serti) were in Hardy-Weinberg equilibrium, while deviation from Hardy-Weinberg equilibrium was recorded at the β-LG locus.

INTRODUCTION

Undoubtedly, the 21st century has witnessed a spectacular amelioration in terms of scientific discoveries. This holds for the agricultural sector, particularly in small ruminant breeding, which is regarded as the most common farm animal in developing countries. Goats were spread along the equatorial areas to the coldest climatic regions. Consequently, goat breeding in Tunisia, a North African country, is important for agricultural holdings; it plays a vital role on the scale of the national economy with more than 1.3 million reproductive females. Goats extend throughout the country, but arid regions include more than sixty percent of the national herd. These domesticated goats are raised under restrictive and irregular natural conditions. The local goat population constitutes a rustic and robust animal population with large variability in terms of morphology and performance[1]. There are several breeds reared in different ecological zones. Depending on climate conditions, these breeds are managed in different production systems. Several works have been devoted to studying the production and reproduction parameters of Iniguez[2], Gaddour *et al*.[3], Ammar *et al*.[4] and Atoui *et al*.[5]. These studies will enable the improvement and creation of new genotypes and genetic heritage conversations. Genetic polymorphisms are an efficient way to evaluate and quantify the genetic diversity of local goats. This phenomenon is very common in selected animal species, which reflects the high variability of the living world, which is always in evolution. While using molecular biology techniques allows easy detection of genetic polymorphisms at the DNA level, it is also possible to analyze their expression at the level of the protein encoded by the gene. Within this frame of reference, this paper has as its objective the study and assessment of genetic variability in the goat population of southwestern Tunisia. This study was realized through genetic polymorphisms of some lactoproteins, including beta-lactoglobulin and kappa casein.

MATERIALS AND METHODS

Presentation of the study area: This study was conducted in Tunisian oasis regions (Jerid and Nefzawa). This region represents 80% of Tunisian oases.

The Jerid region located in southwestern Tunisia is a barren area. It is an intermediate area located between the salty el Gharsa shott and the endorheic basin of Jerids hott. It includes six delegations: Tozeur, Nafta, Degueche, El Hamma Jerid, Tamerza and Hezoua. The region corresponds to the territory of the governorate of Tozeur surrounded to the north by the governorate of Gafsa, to the west by the Algerian border, to the south and the east by the governorate of Kebili. Bounded by coordinates 33°52 and 34°19 North latitude and 7°52 and 8° 24 West longitude.

Nefzawa geographically corresponds to the region bounded by coordinates 33°30 and 34°15 North latitude and 8°30 and 9°10 East longitudes. It extends to the north to the north slopes of Jebel Chareb and Segui to Jebel Berda, to the west to Shott el Jerid, to the south to the great Erg Oriental and to the east the Dhahar plateau. This governorate comprises the delegations of Kebili, Douz, Souk Lahad, Jemna and El Faouar. It corresponds to governorate of Kebili sector surrounded to the northwest by the governorates of Tozeur and Gafsa, to the southeast by the governorates of Tataouine and Medenine and the east by the governorate of Gabes.

Animal samples: The present study focused on the genetic polymorphism of milk proteins in the local goat population exploited in the oasis regions of Tunisia. This population was divided into two subpopulations, Arbi and Serti, highlighted in previous works[6-10].

Sample collection and genomic DNA extraction: For the most constant and faithful representation of the genetic variability of the studied goat population, samples were taken from unrelated individuals as genetically distant as possible. Taking into consideration these conditions:

- Uncontrolled animals without any identification to minimize the risk of sampling
- Related animals; only 2 samples per herd were taken in each geographic location
- Controlled and identified animals: samples were taken after studying their pedigree

Blood samples were taken according to the rules of Tunisian law on blood sampling from living animals. Treatment of animals was performed following good practices related to blood sampling and according to the national ethical guidelines for animal care and use for scientific purposes recommended by the National School of Veterinary Medicine of Sidi Thabet (ENMV) and the General Direction of the Veterinary Service (DGSV) (Number: CEEA-ENMV 36/21). A total of 171 samples for kappa casein CSN3 protein and a total of 151 samples for beta lactoglobulin (Table 1) were taken from adult

Table 1: Distribution of sampled animals

Région	Localities	BLG	CNS3
Jérid	El HammaJérid	16	22
	Tozeur Ville	15	19
	Degueche	15	21
	Nafta	25	22
	Total of region	71	84
Nefzawa	Fatnassa	30	23
	Souk Lahad	20	20
	Kébili Ville	14	23
	Bechni	16	21
	Total of region	80	87
Overall population		151	171

Table 2: Primers used in PCR-RFLP experiments

Gene	Sequence	Annealing	Product size	Enzyme	References
κ-casein	F : TCCCAATGTTGTACTTTCTTAACATC R : GCGTTGTCCTCCTCTTTGATGTCTCCTTG	54°C	645pb	Hae3	Yahyaoui *et al*.[17]
β-Lg	F : GTCACTTTCCCGTCCTGGGG R : GGCCTTTCATGGTCTGGGTGAGG	60°C	710pb	Sma1	Yahyaoui *et al*.[18]

animals in both regions and belonging to both sexes. Using vacuum tubes with anticoagulant (K_3EDTA) and venoject needles, a total volume of 10 mL of whole blood was drawn directly from the jugular vein. The collected blood was kept in dry ice until being transported to the laboratory. Samples were collected from four groups (Serti Jérid and Arbi Jérid; Serti Nefzawa and Arbi Nefzawa) in El Hamma Jérid, Tozeur, Nafta and Degueche in the region of Jérid and in Bechni, Souk Lahad, Fatnassa and Kébili in the region of Nefzawa.

Genomic DNA was extracted from whole blood using the Blood DNA Preparation kit from JENA BIOSCIENCE according to the manufacturer's instructions. DNA concentration and purity were examined using a UV spectrophotometer at 260 nm and 280 nm. Samples with an OD ratio (A260/A280 nm) between 1.7-1.9 were considered good and used for polymerase chain reaction.

Polymerase chain reaction (PCR): Amplification of exon 4 of the κ-casein gene and exon 7 regions of the β-LG gene was performed using specific primers (Table 2). This gene amplification was carried out in sterilized PCR tubes with a volume of 0.5 mL. In a 25 µL reaction volume containing 2 µL of genomic DNA (~ 100 ng), 2.5 µL of 10 X PCR buffer, 200 µM of each dNTP, 0.25 µM of each primer, 2.5 mM MgCl2 and 1 unit of Taq DNA polymerase (Promega, Madison, USA). The volume was completed with pure water. The amplification reaction, which took place in a programmable thermocycler of the "Biometra, T personal" type, consisted of a predenaturation step at 94°C for 5 min, succeeded by 35 cycles each comprising a denaturation step at 94°C for 1 min, primer hybridization step at 60°C for 1 minute and extension at 72°C for 1 min. The last reaction step consisted of the DNA extension strands at 72°C for 5 min and then cooling to a temperature of 4°C.

Thereafter, amplicons were digested overnight at a temperature of 37°C in a final volume of 15 µL. The reaction mixture contained 7 µL of the PCR product, 1.5 µL of the enzyme buffer, 6 µL of pure water and 0.5 µL of the restriction endonuclease (BioLabs Inc., New England) specific for each protein. The product of the enzymatic digestion was then deposited on a 2% agarose gel to separate them by electrophoresis (100 V for 2 h and 30 min) and was then scanned by a gel doc system (Bio-Rad Gel Doc™ XR+). An appropriate size marker (DNA Ladder) of molecular weight 100 bp was also deposited in the gel to determine the weight of the electrophoretic profiles.

Statistical analysis: Realized electrophoretic profiles were used for the analysis and assessment of molecular polymorphisms. Amplified fragments by each primer were demonstrated in all analyzed individuals and by comparison of all electropherograms. Several population genetic parameters were established based on the interpretation of the determined electrophoretic profiles. Statistical indices and parameters were calculated using the POPGENE program (Version 1.32)[11]. Variability was estimated by allele frequencies, genotypic frequencies and heterozygous levels. The deviation from Hardy-Weinberg equilibrium was assessed by the FIS fixation index of Wright[12].

RESULTS AND DISCUSSION

Genotypes of caprine Beta Lactoglobulin: Beta-lactoglobulin is a major milk protein gene in ruminants, accounting for 60 to 65% of the total whey protein in milk. In sheep, the β-LG gene on chromosome number 3 consists of seven small exons and six introns containing 7379 nucleotides[13]. In goats, β-LG has been allotted to chromosome 11q28[14,15] and to the transcription unit of 4698 bp encoding β-LG, 2148 bp and 1242 bp flanking regions 5' and 3', respectively. Two nucleotide substitutions were found between the genomic and cDNA sequences, both located in the 3' untranslated region [T (gene)/C (cDNA) at gene position 4122 and G (gene)/C(cDNA) at position 4605]. The overall structure of the caprine β-LG gene is similar to that of its sheep and cattle counterparts; the sizes of introns and exons are well conserved[15,16].

β-Lactoglobulin locus exon 7 amplification was assessed in 151 Tunisian oasis goats. The amplified PCR product observed is 710 bp in size. Enzymatic digestion of the PCR product with the enzyme SmaI (Fig. 1) gives three different genotypes, each characterized by a different number and size of the fragment. The homozygous form CC presents three fragments of different sizes, 472, 181 and 50 bp, while the other form TT represents only two fragments, 472 and 231 bp. Finally, the heterozygous form CT represents four fragments of different sizes, 472, 231, 181 and 50 bp (Table 3). The polymorphic site consists of a single

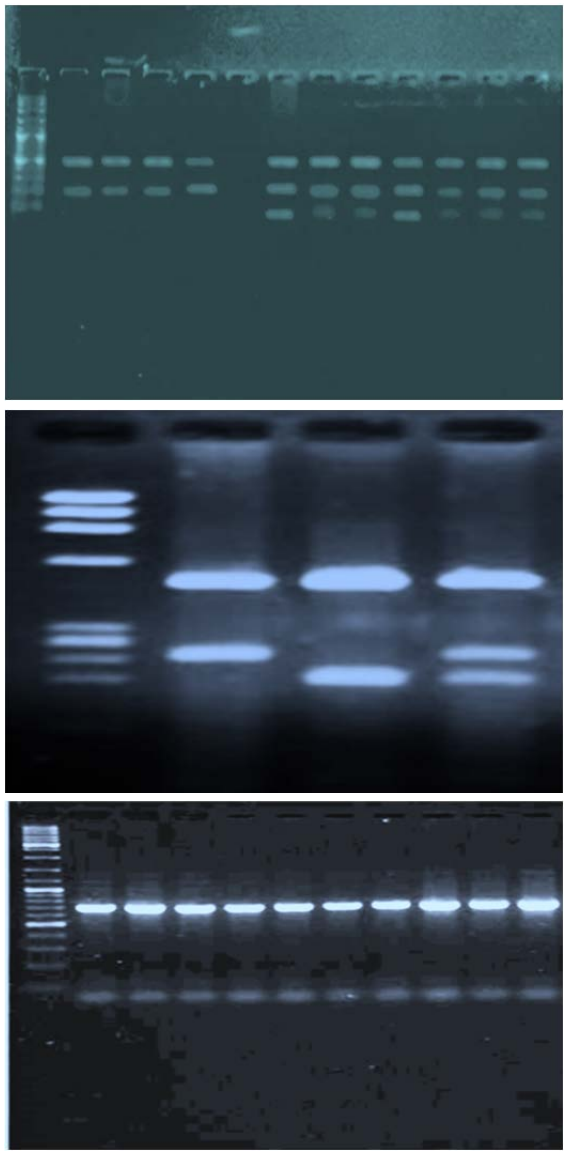

Fig. 1: Sample of PCR-RFLP profile of Beta-lactoglobulin

nucleotide substitution (C to T) at position -60 of the goat β-Lg promoter region. Digestion of the PCR product with the SmaI enzyme of the -60C allele produces three fragments (472, 181 and 50 bp), while digestion of the -60T allele gives only two fragments (472 and 231). The 181 and 231 bp fragments were used for the identification of the -60C and -60T variants and the 472 bp fragment was used for the control of digestion[17].

The study of the β-lactoglobulin protein has shown the existence of two alleles, C and T, in the local goat population in oasis regions. We noticed that the C allele was the most dominant, with a frequency equal to 0.646, while the T allele had only a frequency of 0.354. At the level of the two regions, the same pattern was observed with superiority to the C allele. The two allelic variants detected control three genotypic forms in the population: CC, CT and TT. All three genotypes were present in the entire population and in both study regions (Table 4). The genotypes present different frequencies; in fact, the CC genotype is the most dominant with a frequency equal to 53.5%; therefore, we found the TT genotype with a frequency of 23.43% and the CT genotype with the lowest frequency of 23%. At the regional level, superiority remained in favor of the CC genotype, with 53.3% and 53.7% in Tozeur and Kébili, respectively. For the other two genotypes, the distribution was different. In fact, for the Tozeur region, the heterozygous CT genotype was more frequent than the homozygous TT genotype (26.65% versus 20.11%). Unlike in the Kebili region, we noted a higher frequency of the TT genotype (26.75%) compared to the CT genotype (19.5%).

Table 3: Correspondent genotypes of β-Lg

Genotypes	Molecularweight (Pb)
CC	472
	181
	50
TT	472
	231
	7
CT	472
	231
	181
	50

Table 4: Beta-Lactoglobin genotypic and allelic frequencies

	Genotype			Allele	
Population	CC (%)	CT (%)	TT (%)	C (%)	T (%)
Arbi	54.02	24.61	21.36	65.28	34.72
Arbi (Tozeur)	54.05	27.80	17.72	68.18	31.82
Arbi (Kébili)	54.00	21.48	25.00	62.82	37.18
Serti	53.00	21.50	25.50	63.92	36.08
Serti (Tozeur)	52.60	25.50	22.50	65.79	34.21
Serti (Kébili)	53.40	17.50	28.50	62.20	37.80
Tozeur	53.30	26.65	20.11	67.00	33.00
Kébili	53.75	19.50	26.75	62.50	37.50
Overall population	53.50	23.05	23.43	64.60	35.40

Table 5: Kappa-casein genotypic and allelic frequencies

	Genotype			Allele	
Population	AA (%)	AB (%)	BB (%)	A (%)	B (%)
Arbi	94.60	5.30	0	65.28	34.72
Arbi (Tozeur)	94.34	5.66	0	97.17	2.83
Arbi (Kébili)	95.00	5.00	0	97.50	2.50
Serti	79.50	20.50	0	89.74	10.26
Serti (Tozeur)	83.33	16.66	0	91.67	8.33
Serti (Kébili)	75.00	25.00	0	87.50	12.50
Tozeur	88.80	11.20	0	94.74	5.26
Kébili	85.00	15.00	0	92.76	7.24
Overall population	87.05	12.95	0	93.86	6.14

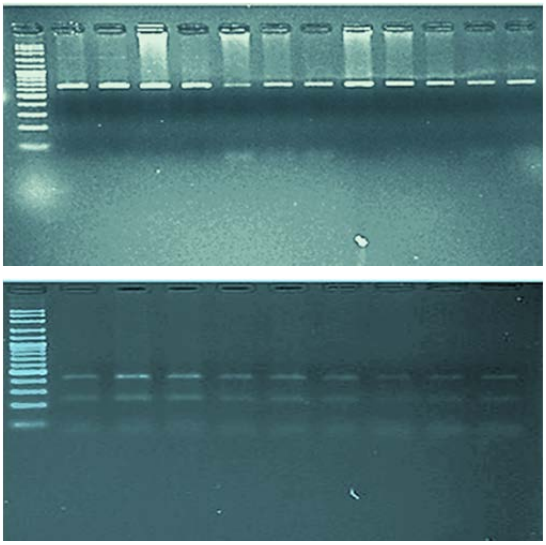

Fig. 2: Sample of PCR-RFLP profile of Kappa casein

Genotypes of caprine Kappa casein: Enzymatic digestion of exon 4 with the enzyme HaeIII (Fig. 2) gave two fragments of different sizes: 416 bp and 229 bp, indicating the presence of the AA genotype of kappa casein in the population. The polymorphic site results from the substitution of only one nucleotide (A with G) at position 242 of exon 4 and produces an amino acid substitution Asp/Gly. The AB genotype (645/416/229 bp) was also detected, while the BB genotype was completely absent in the oasis goat population. Currently, 16 variants of the caprine kappa casein CNS3 locus have been identified by applying 15 polymorphic sites in exon 4 of CNS3[18,19]. In our study, following the enzymatic digestion of exon 4, amplified by endonuclease HaeIII, two alleles were detected in the local goat population, A and B, resulting in the three genotypes AA and AB and AA with a dominance of allele A, which presented 93.86%.

At the regional level, we noticed that in the Tozeur area, the most dominant genotype was AA, with a frequency of 88.8%, whereas genotype AB had only 11.2%. In the Kebili region, the AA genotype also had the highest frequency, which was 85%. The AB genotype had the lowest frequency (Table 5).

The superiority of homozygous genotype AA over heterozygous genotype AB has always been observed in both subpopulations.

Indeed, the frequency of the AA genotype in the Arbi or Serti population, regardless of the study region, is always higher than that of the AB genotype. This superiority was less weak when comparing the Arbi subpopulation with the Serti subpopulation (94.6% versus 79.5% for the AA genotype and 5.3% versus 20.5% for the AB genotype) (Table 5).

This superiority was also observed in the frequencies of the A allele over the frequency of the B allele in the two subpopulations. When comparing the two subpopulations, we noticed that the frequency of the A allele in the Serti group (89.74%) was higher than that of the A allele in the Arbi group (65.28%), whereas for the B allele, the Arbi goats had a higher frequency (34.72%) than Serti animals (10.26%) (Table 5).

Heterozygosity: Heterozygosity is a parameter of genetic diversity used to estimate the genetic variability of a given population. The corresponding results are gathered in Table 6. These results show that the observed heterozygosity is always lower than the expected heterozygosity. Indeed, the observed heterozygosity varies from 0.13 (Arbi subpopulation) to 0.21 (Serti subpopulation), with an average of approximately 0.17. The expected heterozygosity is between 0.25 (Arbi) and 0.32 (Serti), with an average for the entire population equal to 0.29.

At the studied loci, the highest heterozygosity value was recorded for β-LG (0.22). The observed heterozygosity was superior to that expected at the CNS3 locus.

Based on multiple loci, the expected heterozygosity (0.29) was greater than that observed (0.17) for the total population, thus reflecting a positive difference that suggests a deficit in heterozygosity in the population.

Table 6: Observed (Ho) and expected (He) heterozygosity of the local goat population

Locus	Ho	He*
CNS3	0.1228 ± 0.06	0.1156 ± 0.21
β-LG	0.2185 ± 0.05	0.4591 ± 0.23
Arbi	0.1380 ± 0.12	0.2545 ± 0.28
Serti	0.2102 ± 0.01	0.3247 ± 0.20
Overall population	0.1707 ± 0.07	0.2873 ± 0.24

Table 7: χ^2 test for the deviation of the Hardy-Weinberg equilibrium

Locus	Arbi	Serti	Total
β-LG	***	***	***
CNS3	NS	NS	NS

NS: Not significant, *p<0.05, **p<0.01, ***p<0.001

Hardy-Weinberg equilibrium: The deviation of the frequencies of observed genotypes from those of expected genotypes under Hardy-Weinberg equilibrium was tested by χ^2 for each subpopulation and for each locus.

Table 7 shows the results of this test. at the CNS3 locus, the total population and the two subpopulations (Arbi and Serti) appear to be in Hardy-Weinberg equilibrium.

A highly significant deviation (p<0.001) from Hardy-Weinberg equilibrium was recorded at the β-LG locus for the entire local goat population.

DISCUSSION

Beta lactoglobulin gene variation: β-Lactoglobulin genetic polymorphisms are important and well known because of their effects on the quantitative traits and technological properties of milk[20-22]. Thus, we studied the β-lactoglobulin of local goat populations with the PCR-RFLP technique for genetic evaluation. Ahmed and Othman, in their study on goat β-lactoglobulin genotypes, amplified a 710-bp fragment and detected three variants, TT, TC and CC, after digestion of the PCR products by the SmaI enzyme. The same trend was also noticed in our study results with three variants. The CC genotype was the most dominant, with 53.5%, followed by the TT and CT genotypes, with frequencies of 23.43 and 23%, respectively. Cardona *et al*.[16] cited three genotypes, namely, AA, AB and BB, in Colombian goats. Additionally, Cardona *et al*.[16] noticed that β-lactoglobulin genotype AA showed greater dairy performances than the BB genotype for all traits along the whole lactation curve, while heterozygotes exhibited moderate yield.

Allele structure of the beta-lactoglobulin (β-LG) gene in the Hair and Halep (Damascus) breeds grown in Turkey[19] with two alleles (A and B) and two genotypes (AA and AB). The BB genotype does not occur in the Hair breed or the Halep breed. The genotype frequencies of the B allele were 0.721 and 0.98 for AA and 0.279 and 0.052 for AB, respectively.

There were conflicting reports regarding the association of β-LG variants with milk yield and composition[20,21]. El Hanafy et al.[22] found a relationship between the exon 7 β-LG gene polymorphism and milk yield in three local Egyptian goat breeds. According to them, genotype β-LG AA gives a higher milk yield than genotype β-LG AB. However, they did not find a significant relationship between the identified genotypes and the components of milk, except for protein percentage. Gharedaghi *et al*.[23] reported that the polymorphism of exon 7 of the β-LG gene was associated with a high milk protein ratio in the Mahabadi goat breed. However, this polymorphism was not significant for milk yield or milk fat percentage. Similar results were cited by El Shazly *et al*.[13], who found that the β-LG genotype had no significant effect on milk yield or milk composition. Except for milk protein content, which was abundant in the β-LG genotype in sheep breeds from Saudi Arabia.

In general, the resemblance between the nearby species (goats and sheep) is higher. However, the similarity obtained in goat-cow comparisons of the 3' untranslated and 3' flanking regions was higher than those calculated in comparisons in which the ovine sequences were included. Most sequence differences in the ovine 3' flanking region occur between positions 5025-5234 and are mainly due to gaps of one or several nucleotides[14]. Jawasreh *et al*.[24] stated that the β-lactoglobulin gene frequency had a greater prevalence of the B allele (0.58). BB and AB were predominant in the Jordanian ovine population, with frequencies of 0.32 and 0.51, respectively.

In addition, Anggraeni *et al*.[25] reported that for the β-lactoglobulin gene in Indonesian cattle, enzyme Hae III cut at two base sites at positions of 74 and 148 bp of PCR products, resulting in three fragments of 74 bp, 74 bp and 99 bp, expressed as the BB genotype. The identified mutation type was a substitution mutation for the base changes from purine (AG) or pyrimidine (TC) into cytosine or thymine.

Akter *et al*.[26] stated that for the β-lactoglobulin gene, the BB genotype (0.66) was found more frequently than the AB (0.18) and AA (0.16) genotypes. The highest frequency was found for B (0.75), followed by the A allele (0.25).

Kappa casein gene variation: Previous genetic identification of different variants of the kappa casein in the Tunisian goat population[27] stated that the kappa casein allelic variants in tested animals revealed different genotypes; two were homozygous: AA or BB, AC or BC and CC. their genotypic frequencies were 12.5, 27 and 60.5%, respectively.

Comparable results were obtained by Othman and Ahmed[19] in their exploration of three primers, 13F, kb1

and kb2, with two restriction enzymes (Alw44I and HaeIII) for genotyping k-CN exon 4. The resultant 645 bp PCR fragment with primers kb2 and 13F was digested with HaeIII to detect alleles A and E. Additionally, they revealed that the B allele was the most frequent in the four studied breeds and displayed a frequency of 68.2%, whereas the C allele had a frequency equal to 22.7%. Allele A was expressed with different frequencies ranging from 10 to 45.5%.

In the Sardinian goat in Italy, Vacca *et al*.[28] noted the dominance of alleles CSN3 A and B; the presence of rare alleles such as CSN3 B", C, C', D, E and M; and the CSN3 S allele (GenBank KF644565) described for the first time in Capra hircus[36]. Furthermore, a total of five polymorphic sites have been described by Singh et al.[29] at the κ-casein (CSN3) locus in the Indian domestic Gaddi goat (Capra hircus).

Nevertheless, the k-casein gene in Girgentana dairy goats, exon 4 of the k-casein (CSN3) gene, was sequenced and analyzed. The resulting sequence analyses showed the presence of known A, B, D and G alleles and two new genetic variants called N and D'. The new D' allele differs from D in one transition, G284→A284, which did not cause amino acid changes[30].

In cattle breeds, κ-casein genotyping was performed using restriction fragment length polymorphism (RFLP) after polymerase chain reaction (PCR)-based amplification of genomic DNA. Allele frequencies were 0.39 for the A-allele and 0.61 for the B-allele. Genotype frequencies were 0.09, 0.60 and 0.31 for genotypes AA, AB and BB, respectively. The κ-casein genotype in tropical milking Criollo cows did not affect milk yield or composition[31].

Heterozygosity and Hardy-Weinberg equilibrium: The observed and expected heterozygosity and P value associated with the null hypothesis of HWE were estimated. A significant departure from HWE was observed for the Arbi and Serti goat subpopulations at the β-lactoglobulin locus, whereas the local population was in Hardy-Weinberg equilibrium at the CSN3 locus ($p<0.05$). Deviation from HWE at the β-lactoglobulin locus is probably because of heterozygote deficiency (Ho = 0.17 vs He = 0.29)[32]. This heterozygous deficit is certainly because of the logical consequence, as announced by Menrad *et al*.[33] of the current high inbreeding and selection against heterozygotes existing in the goat population reared in southwestern Tunisia. Indeed, animals raised with a relatively small number per herd and breeder are characterized by the marked use of the same goat for several years and even if it is excluded from the herd, one of these descendants takes its place in the breeding system[2].

Studying the Girgentana goat breed at the CSN3 locus, Di Gerlando *et al*.[30] observed a significant departure from HWE ($p<0.05$), probably due to heterozygote excess (Ho = 0.6766 vs He = 0.6243).

Deflection from Hardy-Weinberg equilibrium outlined the β-LG polymorphism of two Turkish goat varieties[21], mainly in the Hair goat breed ($p<0.05$), while deflection from Hardy-Weinberg equilibrium was not observed in the Halep goats.

The average genetic diversity (He) was 0.38. The results indicated differences between observed (Ho) and expected (He) heterozygosity and it was out of equilibrium genetics, assuming that selection pressure was in the population[26].

CONCLUSION

This study contributes to the knowledge and investigation of the local goat population in southwestern Tunisia since it is interested in the genetic polymorphism of two milk proteins, beta lactoglobulin and kappa casein, through molecular methods. Allelic and genotypic frequencies were estimated, in addition to the observed (Ho) and expected (He) heterozygosity frequencies of different loci. Sample analysis of the local goat population indicated the presence of two alleles A and B and two genotypes AA and AB for the kappa casein protein as well as two alleles C and T and three CC genotypes TT and CT for the β-LG protein via the PCR-RFLP technique, while their allelic frequency and genotypic heterozygosity varied by region (Jerid and Nefzawa) and according to the subpopulation (Serti and Arbi).

Furthermore, this work allowed us to conclude that in the whole population, the deviation of the Hardy-Weinberg equilibrium was not significant for the CNS3 locus, while it was highly significant for the β-LG locus. Consequently, this genetic identification of Tunisian goat milk protein will enable the establishment of a better strategy for Tunisian goat breeding, contributing to satisfying the nutritious gap in milk production.

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The blood samples were taken according to the rules of the Tunisian law approving blood sampling from living animals in Official Gazette of the Republic of Tunisia

number 28 (Decree No. 2011-400 of April 18, 2011; APBvet 10: Samples from live animals: blood collection, organs, swabs). Animals were handled after getting permission from the competent authority (The regional public veterinary services at the Regional Commissariat for Agricultural Development (CRDA) in Tozeur and Kébili) and in the presence of herd owners. Treatment of animals was performed following good practices related to blood sampling and according to the national ethical guidelines for animal care and use for scientific purposes recommended by the National School of Veterinary Medicine of Sidi Thabet (ENMV) and the General Direction of the Veterinary Service (DGSV) (Number: CEEA-ENMV 36/21). Blood samples were taken by two people, a technician to immobilize the animal and an official and experienced veterinarian to bleed the animal. Immediately after the withdrawal of the needle, a firm pressure was applied to the venipuncture site for ten to fifteen seconds. Once the blood has been removed from the animal, the insertion site was swabbed with alcohol to remove any bacteria that might have entered the area during the drawing of the blood. The sampled animals were watched closely for an hour or two to ensure that no bleeding occurs and that the animal exhibits normal behavior. Used needles were properly disposed of in a sharps container for return to the veterinarian for disposal.

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