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Effect of Kappa Casein and Beta Lactoglobulin Genetic Variants on Milk Composition Traits in Tunisian Oasis Autochthonous Goats

Zahran Khaldi[1*], Mounir Nafti[1], Mohamed Tabarek Jilani[2], Sami Souid[3]

[1]Department of Animal Production, Regional Center for Research in Oasis Agriculture, Institution of Agricultural Research and Higher Education, University of Carthage, Route Tozeur Km1, Degueche, Tunisia.

[2]Regional Public Health Laboratory of Tozeur, Ministry of Public Health, 2233, Bled El hadar, Tozeur, Tunisia.

[3]Research Unit of Macromolecular Biochemistry and Genetics, Faculty of Sciences of Gafsa, University of Gafsa, 2112, Gafsa, Tunisia.

***Correspondence**
Zahran Khaldi
Department of Animal Production, Regional Center for Research in Oasis Agriculture, Institution of Agricultural Research and Higher Education, University of Carthage, Route Tozeur Km1, Degueche, Tunisia.
E-mail address: khaldi_zahrane@yahoo.co.uk

Abstract

The undertaken study was planned to investigate the influence of genetic variants of β-lactoglobulin and κ-casein loci on milk composition traits in two autochthonous Arbi and Serti goats raised in Tunisia's continental oasis region. For this purpose, blood and milk from 177 multiparous and unrelated animals were collected and used. Milk samples were analyzed for physical parameters, chemical composition, and mineral concentrations using standard methods. PCR amplification was performed using DNA samples and specific primers targeting exon 7 and 4 of β-lactoglobulin and κ-casein, respectively. To detect genetic variants, amplified products were digested with the restriction enzymes SmaI for β-lactoglobulin and HaeIII for κ-casein. The κ-casein protein had two alleles A and B, as well as two genotypes AA and AB, whereas the β-lactoglobulin showed two alleles C and T, and three genotypes CC, TT, and CT. Allele A of κ-casein occurred at a higher frequency than allele B in Arbi and Serti subpopulations and overall population. The β-lactoglobulin C allele was more common than T allele in Arbi and Serti subpopulations and throughout population. Homozygotes' frequencies were found to be higher than those of heterozygotes in all animals. Genetic polymorphism of β-lactoglobulin has been linked to milk acidity, dry matter, protein, casein, fat, lactose, Ca, P, and k content, with homozygous TT and CC genotypes clearly outperforming CT genotype. The κ-casein locus had a marked influence on the dry matter, protein, casein, and fat components, favoring homozygous AA goats in all traits except fat, which distinguished heterozygous AB goats.

KEYWORDS
Autochthonous goat, Polymorphism, Milk composition, Variation, β-lactoglobulin, κ-casein.

INTRODUCTION

Goat breeding is one of Tunisia's most traditional activities, with a significant social, cultural, and economic impact. It has always existed primarily to exploit the mountainous and arid regions of the country's center and south. The national herd of 1197090 head, including 564,330 in the south of the country (DGEDA, 2020), is essentially composed of the rustic native local population. The local goat is raised mainly under fragile production systems reliant on exogenous inputs and a diet based on spontaneous natural plant resources that are highly vulnerable to climatic hazards. Zootechnical performances in goats are mainly affected by nutritional, physiological, environmental factors, and the genetic potential of animals. Although many studies have been conducted in Tunisia on the genetic characterization of native goats (Ouni *et al*., 2007; Vacca *et al*., 2009; Jemmali *et al*., 2013; Ben Zaabza *et al*., 2015; Nafti *et al*., 2016), little is known about the relationship between productive traits and their genotypes. The few available data only relates to cattle species in terms of reproductive traits (Amiri *et al*., 2018), the prevalence of lameness (Ferchichi *et al*., 2018), tuberculosis resistance (Bejaoui *et al*., 2021), and prolificacy and female sterility in Barbarine sheep breed (Lassoued *et al*., 2017). Domestic animals can be genetically improved using molecular genetic markers (Yang *et al*., 2013), and recent advances in molecular genetics have resulted in the identification of individual genes or candidate genes with significant effects on economically important traits (Gebreselassie *et al*., 2020).

The analysis of genetic variants involved in productive traits in farm animals is the first step toward increasing profitability. It should not be forgotten that often, in the same species, with the same environmental effects, as the breeds vary, a certain level of productivity can be associated with different polymorphisms in the same genes or with completely different genes (Albarella *et al*., 2020). This is the reason why we need to not only verify the genotype of a livestock breed of interest but also to correlate it with production performances. Native breeds are of great interest because they are important reservoirs of valuable genetic variability, which may be targeted by conservation efforts (Zorc *et al*., 2022). Moreover, they might possess unique genetic variations that can affect the milk composition and quality, potentially resulting in distinct milk characteristics that could be exploited in future breeding programs (Albarella *et al*., 2020). Several studies have been conducted around the world to study the effect of lactoprotein genetic variants on milk qualitative and quantitative traits (Caravaca *et al*., 2011; Yousefi *et al*., 2013; Cardona *et al*.,

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2016; Catota-Gómez *et al*., 2017; Jawasreh *et al*., 2019; Kyselová *et al*., 2019; Albarella *et al*., 2020); however, there is a lack of relevant research linking gene polymorphism to quantitative and qualitative milk traits in local animal genetic resources, which justifies investigations on these genes.

The present study screens some native goat genetic loci for possible variants in the β–lactoglobulin and κ–casein genes and establishes their frequency in the Serti and Arbi subgroups with the ultimate objective of investigating the effect of these genotypes on physical parameters, chemical composition, and mineral concentrations of their milk.

MATERIALS AND METHODS

The current study was conducted in the governorates of Tozeur and Kebili, which are in the continental oases area of Tunisia's southwest. This region contains 80% of Tunisia's oases and is part of the Saharan domain, which has an arid to the hyper-arid bioclimatic stage. With an annual total rainfall of less than 100 mm, precipitation is infrequent and irregular. A highly contrasting thermal regime with large annual and diurnal amplitudes reflects the region's pronounced continentality. The average temperature is approximately 22°C, with extremely high summer temperatures (a maximum of 55°C in July).

Animals and management conditions

The two subpopulations, Arbi and Serti belonging to the local goat population highlighted in previous works (Nafti *et al*., 2009; Nafti *et al*., 2013; Nafti *et al*., 2014; Ben Zaabza *et al*., 2015; Nafti *et al*., 2016) and raised in the oasis region are used in the present study. Healthy multiparous animals from private flocks having uniform body conditions, aged 4 years, at the 3rd lactation, and the mid-lactation stage were selected for blood and milk sampling. Goat subgroups were managed under a traditional herding system; wither goats grazed during the daytime for 6-7 h on natural pasture in the oases' vicinity. In addition to pasture, and regardless of their physiological stage, animals were fed barley, wheat bran, downgraded dates, and green Medicago sativa. The chemical compositions were [DM: 91%; CP: 12.7%; NDF: 26.3%; Ash: 3.9%; Net energy content: 1817.01 kcal. kg-1DM], [DM: 86.92%; CP: 14.9%; NDF: 37.7%; Ash: 4.1%; Net energy content: 1624.97 kcal. kg^{-1} DM], [DM: 88.56%; CP: 3.11%; NDF: 17.81%; Ash: 2.69%; Net energy content: 1931.42 kcal. kg^{-1} DM], [DM: 28.66%; CP: 19.22%; NDF: 45.3%; Ash: 14.20%; Net energy content: 1320 kcal. kg^{-1} DM] for barley, wheat bran, dates, and lucerne, respectively (Khaldi *et al*., 2022). Goats were kept with their kids during the day and the evening containment period, except on days when milk samples were collected.

Samples collection

A total of 177 unrelated animals distributed over the two study governorates were sampled. They included milk and blood samples collected from 94 Arbi and 83 Serti goats. Fresh milk samples were collected in the morning by direct milking from complete milking. A duplicate individual sample of 300 ml from healthy animals was collected in sterile tubes and kept in an ice container during sampling and transportation to the laboratory for physicochemical and mineral analysis. Whole blood was drawn directly from the jugular vein into sterile 10 mL vacuum tubes containing the K_3EDTA anticoagulant. Blood samples were subsequently stored at -20°C until further use.

Ethical approval

This study was reviewed and approved by the specialized experimentation unit of the Regional Research Center of Oasis Agriculture. Consent was obtained from owners for their animal's participation in this study.

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).

Milk composition analysis

All physical parameters were determined on the same sampling day. A CONsort C933 pH meter was used to measure the pH at 20°C. To determine milk density at 20°C, a Gerber thermo-lacto-densimeter was used. Dornic acidity was determined using the AOAC-recommended titrimetric method (AOAC, 2000). The milk samples were analyzed for lactose and ash using the official international analytical methods AOAC (2005) and AOAC (2012), respectively. Fat (IDF, 2009), dry matter (IDF, 2010), and total protein (IDF, 2014) were determined according to the IDF standard methods. The casein content was obtained by the difference between total nitrogen and non-casein nitrogen using the Kjeldahl method (IDF, 2004). Pursuant to method of IDF (2007) and using an atomic absorption spectrophotometer (Analytikjena: nova 400), calcium was measured. Jenway flame emission spectroscopy has been used to determine sodium and potassium in compliance with AOAC standards (1984). The phosphorus present in the milk sample was measured according to GB (2010).

Genomic DNA extraction and PCR-RFLP analysis

DNA extraction was carried out using two DNA purification Kits, the "Wizard® Genomic DNA Purification Kit" (Promega Corporation, Madison, USA) and the "Blood DNA Preparation Kit" (Jena Bioscience, Loebstedter, Jena, Germany) on 3 ml of total blood according to the manufacturer's protocol. DNA concentration and purity were determined using a UV spectrophotometer at 260 and 280 nm optical densities. The quality of DNA was also verified on 0.8% agarose gel electrophoresis. Only good samples were considered and used for polymerase chain reaction. β-lactoglobulin and κ-casein genotypes were typed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The DNA samples were amplified through the polymerase chain reaction method, using specific primers targeting exon 7 of β–lactoglobulin and exon 4 of κ–casein as shown in Table 1. The amplification reaction was performed in a final volume mixture of 25 µl 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 $MgCl_2$, and 1 unit of Taq DNA polymerase (Promega, Madison, USA). MilliQ sterilized water was added up to the final volume. The PCR reaction was carried out using Bio-Rad T100

thermal cycler in the following programs of initial denaturation step at 94°C for 5 minutes, followed by 35 cycles each comprising a denaturation step at 94°C for 1 minute, primer hybridization step at 54-60°C for 1 minute and extension at 72°C for 1 minute. The last reaction step consisted of the DNA extension strands at 72°C for 5 minutes and then cooling to a temperature of 4°C.

The PCR products 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 studied gene (Table 1). The restriction products were separated in agarose gel at a concentration of 2% in presence of 100 bp DNA ladder as a molecular size marker and stained with ethidium bromide. Electrophoresis patterns were scanned and documented using a gel doc system (Bio-Rad Gel Doc™ XR+) for allele discrimination.

Statistical analysis

Allelic and genotypic frequencies of β–lactoglobulin and κ–casein were calculated using POPGENE program (Version 1.32) (Yeh *et al*., 1999). A statistical analysis was carried out to estimate the effect of studied gene genotypes on milk composition traits. The GLM procedure, implemented with SAS software (2004), included a fixed effect of the loci genotypes, subgroup, location, and herd. Differences between the two subgroups were performed by comparing the averages of different studied traits using the Tukey–Kramer test. The statistical model was used as described below:

$Y_{ijkl} = \mu + S_i + G_j + L_k + Hl + e_{ijkl}$

Where Y_{ijkl} is the observation of each studied trait (pH, acidity, density, dry matter, protein, fat, casein, lactose, ash, Ca, P, Na, K); µ: is the general mean of each trait; Siis the fixed effect of ith subgroup (Arbi and Serti); G_j is the fixed effect of the jth genotype at the studied locus (β–lactoglobulin = CC, CT, and TT; κ–casein = AA and AB); L_k is the fixed effect of Kth locality (Tozeur and Kebili); Hl is the fixed effect of the lth herd; e_{ijk} is the random error.

RESULTS

Milk composition

The milk gross composition of the local goat population is presented in Table 2. Milk's average physical properties, including pH, acidity, and density were 6.54±0.09, 15.88±2.10, and 1030.99±2.78, respectively. Significant differences ($P < 0.05$) were found between the two subgroups on the mentioned parameters, favoring Arbi goats. The same trend has been observed for chemical components and mineral element concentrations except for the higher fat content ($P < 0.05$) in Serti Milk. At the total population level, the chemical compositions were 133.77±24.59, 35.35±6.04, 42.28±6.91, 28.51±4.73, 40.38±5.55, and 7.91±1.69 g/L for total solids, protein, fat, casein, lactose, and ash, respectively. The overall mineral concentrations vary between 0.27±0.08 and 1.48±0.32 g/L.

Genotypic and allelic frequencies of milk protein variants

The amplification of the β-lactoglobulin locus exon 7 tested in 151 Tunisian oasis goats revealed that the size of the amplified PCR product observed was 710 bp. Digestion of the PCR product with the specific enzyme (Sma I) generated three different genotypes, each with a different number and size of fragments. The homozygous form CC presents three fragments of varying sizes, 472, 181, and 50 bp, whereas the TT form has only two fragments, 472 and 231 bp. Finally, the heterozygous form CT consists of

Table 1. Primers and restriction enzymes used in PCR-RFLP experiments.

Gene	Sequence (5'→3')	TM (°C)	Product size (pb)	RE	References
κ-casein	F: TCCCAATGTTGTACTTTCTTAACATC R: GCGTTGTCCTCCTCTTTGATGTCTCCTTG	54	645	Hae3	Yahyaoui *et al*. (2003)
β-Lg	F: GTCACTTTCCCGTCCTGGGG R: GGCCTTTCATGGTCTGGGTGAGG	60	710	Sma1	Yahyaoui *et al*. (2000)

TM: Annealing temperature; RE: restriction enzyme used for Restriction Fragment Length Polymorphism (RFLP).

Table 2. Summary of milk composition traits (mean ± standard deviation) in autochthonous goats.

Traits \ Subgroup	Arbi (n.=93)	Serti (n.=83)	Total population (n.= 176)
pH	6.57±0.11[a]	6.50±0.05[b]	6.54±0.09
Acidity	16.20±1.97[a]	15.43±2.15[b]	15.88±2.10
Density (°D)	1030.27±2.35[a]	1031.80±3.01[b]	1030.99±2.78
Dry matter (g/L)	151.14±19.81[a]	116.40±16.49[b]	133.77±24.59
Protein (g/L)	38.24±5.21[a]	32.44±5.76[b]	35.35±6.04
Fat (g/L)	39.72±5.68[b]	45.69±6.59[a]	42.28±6.91
Casein (g/L)	30.49±4.17[a]	26.56±4.71[b]	28.51±4.73
Lactose (g/L)	41.27±5.47[a]	39.37±5.50[b]	40.38±5.55
Ash (g/L)	8.27±1.67[a]	7.52±1.64[b]	7.91±1.69
Ca (g/L)	1.46±0.30[a]	1.35±0.30[b]	1.41±0.30
P (g/L)	1.27±0.28[a]	1.13±0.25[b]	1.20±0.27
Na (g/L)	0.33±0.07[a]	0.21±0.04[b]	0.27±0.08
K (g/L)	1.53±0.32[a]	1.41±0.31[b]	1.48±0.32

[a, b, c] values with different superscripts within the same column are significantly different ($p<0.5$).

four fragments of differing sizes: 472, 231, 181, and 50 bp. In the entire population (Table 3), 53.5% were genotyped as CC, 23.05% as CT, and 23.43% as TT. Thus, the allelic frequencies were 64.6% and 35.4% for C and T alleles, respectively. At the subgroup level, the CC genotype was more prevalent in both Arbi and Serti goats. The distribution of the other two genotypes was different between the studied subgroups. The heterozygous CT genotype was more frequent in Arbi subgroup than the homozygous TT genotype. Unlike for the Serti goats, the TT genotype was found to be more common (25.32%) than the CT genotype (21.52%).

At the level of the two regions, the same pattern of allelic frequency was observed with superiority to the C allele. The CC genotype remained more frequent, 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%).

Regarding the κ-casein locus, the size of the PCR-amplified fragment was 645 bp for 171 animals. Enzymatic digestion of amplified product by HaeIII restriction endonuclease revealed two alleles (A and B) and two genotypes with different sizes designated as homozygous AA (416 and 229 bp) and heterozygous AB (645, 416, and 229 bp). BB genotype was absent throughout the studied animals. The frequency overall population of Allele A and B at κ-casein locus (Table 3) were 93.86% and 6.14%, respectively resulting in the AA genotype being the most frequent (87.05%) compared to the AB genotype (12.95%). Within subgroups, the dominance of allele A and genotype AA is often observed, regardless of the study region.

Effect of genetic variants on milk composition traits

The results of analysis of effects of β-lactoglobulin and κ-casein variants on milk physical characteristics are summarized in Table 4. The data indicate that β-lactoglobulin alleles and genotypes had no effect (P > 0.05) on pH and density of milk overall population and subgroups. Similarly, milk acidity was not affected by the β-lactoglobulin alleles within the Arbi subgroup, in contrast to the Serti subgroup and total population (P < 0.05). Heterozygous Goats (CT) produced milk with a higher acidity than homozygous animals (CC and TT) within the Serti subgroup and overall goat population. No significant relationship was observed between the occurrence of polymorphic forms of the κ-casein gene and the physical characteristics of milk (P > 0.05) within subgroups or the overall population.

In Arbi subgroup, the CC and TT genotypes of β-lactoglobulin gene were associated with the highest contents of total solids, protein, casein, and lactose compared to the CT genotype (P <

Table 3. Allelic and genotypic frequencies (%) of β-lactoglobulin andκ-casein in oases local goats.

	β-lactogloulin					κ-casein			
	C	T	CC	CT	TT	A	B	AA	AB
Subgroup									
Arbi	65.28	34.72	54.02	24.61	21.36	65.28	34.72	94.6	5.3
Serti	63.92	36.08	53.16	21.52	25.32	89.74	10.26	79.5	20.5
Locality									
Tozeur	67	33	53.3	26.65	20.11	94.74	5.26	88.8	11.2
Kebili	62.5	37.5	53.75	19.5	26.75	92.76	7.24	85	15
Total population	64.6	35.4	53.5	23.05	23.43	93.86	6.14	87.05	12.95

Table 4. Effect of β-lactoglobulin and κ-casein genotypes on milk physical characteristics from local goats.

	Genotype	pH	Acidity	Density (°D)
β-lactogloulin				
Arbi	CC	6.58±0.14^{a}	15.92±1.91^{a}	1030.44±2.19^{a}
	CT	6.57±0.06^{a}	16.96±1.98^{a}	1030.38±3.03^{a}
	TT	6.58±0.13^{a}	16.14±2.23^{a}	1030.12±2.08^{a}
Serti	CC	6.49±0.04^{a}	14.92±2.20^{b}	1031.57±3.13^{a}
	CT	6.51±0.05^{a}	16.6±1.99 ^{a}	1033±3.04^{a}
	TT	6.50±0.05^{a}	15.50±1.78^{ab}	1031.45±2.79^{a}
Total population	CC	6.54 ±0.11^{a}	15.40±2.11^{b}	1031.02±2.76^{a}
	CT	6.55±0.06^{a}	16.77±1.96^{a}	1031.73±3.27^{a}
	TT	6.54±0.10^{a}	15.79±2^{b}	1030.84±2.55^{a}
κ- casein				
Arbi	AA	6.57±0.11^{a}	16.30±1.98^{a}	1030.22±2.34^{a}
	AB	6.55±0.12^{a}	16.50±2.09^{a}	1031.2±2.58^{a}
Serti	AA	6.51±0.05^{a}	15.66±2.09^{a}	1031.89±2.88^{a}
	AB	6.48±0.04^{a}	14.62±2.43^{a}	1031.56±3.70^{a}
Total population	AA	6.55±0.09^{a}	16.03±2.04^{a}	1030.91±2.7^{a}
	AB	6.50±0.07^{a}	15.07±2.44^{a}	1031.48±3.41^{a}

$^{a, b, c}$ values with different superscripts within the same column are significantly different (p<0.5).

0.05) (Table 5). The three genotypes showed no significant difference (P > 0.05) in fat and ash content. Inside Serti breed, homozygous TT goats showed statistically significant higher (P < 0.05) total solids, protein, and casein levels than the CC and CT types. Both of homozygous genotypes TT and CC were significantly associated with higher fat content, while CT genotype with the lowest (P < 0.05). However, lactose and ash were not significantly affected by β-lactoglobulin variants (P > 0.05).

Considering the total population, the genotype CT showed the lowest dry matter, protein, fat, casein, and lactose (P < 0.05), whereas homozygous goats (CC and TT) produced milk with more total solids, protein, fat, casein, and lactose (P < 0.05). Concerning the β-lactoglobulin gene, no significant differences (P < 0.05) among detected genotypes were found in ash content.

In terms of κ-casein in Serti goats, the AA genotype had higher protein and casein content than the AB genotype, but the AB genotype outperformed the AA genotype in fat (P > 0.05). When considering the total population, results illustrated a higher dry matter, protein, and casein content and lower fat concentration of AA genotype compared to AB goats. Nonetheless, no significant differences in lactose and ash content among Serti animals or in all chemical components of milk within the Arbi subgroup were found between the κ-casein genotypes (P > 0.05).

Analysis of selected minerals is given in Table 6. Evaluation of the relationship between genetic variants of β-lactoglobulin and mineral concentrations showed that the milk of Serti goats with TT genotype had the largest levels of Ca, P, and K, followed by goats with CC genotypes, then those having the heterozygous CT genotype. However, concentrations of Na were similar (P >0.05) among the three genotypes. These differences were confirmed

Table 5. Effect of β-lactoglobulin and κ-casein genotypes on chemical composition (g/L) of goat milk in Tunisian oases

	Genotype	DM	Protein	Fat	Casein	Lactose	Ash
β-lactogloulin							
Arbi	CC	154.87±16.46^{a}	39.06±4.9^{a}	40.28±5.46^{a}	31.07±3.90^{a}	42.82±4.55^{a}	8.11±1.75^{a}
	CT	142.41±22.9^{b}	34.73±4.33^{b}	39.1±6.27^{a}	28.30±4.16^{b}	39.37±6.33^{b}	8.34±1.74^{a}
	TT	153.79±13.07^{a}	39.67±3.91^{a}	39.02±5.43^{a}	31.24±3.06^{a}	42.52±3.61^{a}	8.38±1.32^{a}
Serti	CC	114.56±15.29^{b}	31.74±5.96^{b}	46.71±6.04^{a}	25.99±4.88^{b}	38.79±5.15^{a}	7.74±1.30^{a}
	CT	112.69±16.83^{b}	30.74±5.63^{b}	39.78±7.29^{b}	25.19±4.63^{b}	38.11±5.71^{a}	6.92±2.04^{a}
	TT	120.81±17.89^{a}	35.37±4.81^{a}	48.57±4.39^{a}	28.91±3.91^{a}	40.94±6.01^{a}	7.66±1.78^{a}
Total population	CC	133.97±25.68^{ab}	35.26±6.57^{a}	43.61±6.58^{a}	28.43±5.09^{a}	40.73±5.25^{ab}	7.92±1.53^{a}
	CT	127.10±24.8^{b}	32.67±5.36^{b}	39.45±6.72^{b}	26.70±4.61^{b}	38.72±5.96^{b}	7.61±2^{a}
	TT	135.96±22.86^{a}	37.35±4.87^{a}	44.18±6.82^{a}	29.98±3.69^{a}	41.66±5.05^{a}	7.99±1.61^{a}
κ- casein							
Arbi	AA	148.66±20.05^{a}	37.70±5.21^{a}	39.11±5.77^{a}	30.06±4.18^{a}	41.10±5.54^{a}	8.30±1.69^{a}
	AB	160.04±11.45^{a}	39.41±5.58^{a}	41.18±3.54^{a}	31.05±4.13^{a}	44.25±3.16^{a}	7.68±1.33^{a}
Serti	AA	116.29±15.53^{a}	33.07±5.38^{a}	44.87±6.82^{b}	27.06±4.39^{a}	39.38±5.24^{a}	7.49±1.64^{a}
	AB	113.65±19.13^{a}	29.45±6.15^{b}	47.24±5.72^{a}	24.14±5.04^{b}	38.29±6.13^{a}	7.61±1.89^{a}
Total population	AA	135.28±24.27^{a}	35.79±5.74^{a}	41.49±6.83^{b}	28.82±4.51^{a}	40.39±5.47^{a}	7.97±1.71^{a}
	AB	124.69±26.66^{b}	31.82±7.31^{b}	45.8±5.83^{a}	25.78±5.62^{b}	39.71±6.07^{a}	7.62±1.74^{a}

$^{a, b, c}$ values with different superscripts within the same column are significantly different (p<0.5).

Table 6. Effect of β-lactoglobulin and κ-casein genotypes on milk mineral contents (g/L) in Arbi and Serti goats

	Genotype	Ca	P	Na	K
β-lactogloulin					
Arbi	CC	1.44±0.31^{a}	1.21±0.26^{b}	0.33±0.07^{a}	1.52±0.32^{a}
	CT	1.43±0.29^{a}	1.22±0.25^{b}	0.34±0.07^{a}	1.45±0.34^{a}
	TT	1.55±0.24^{a}	1.47±0.32^{a}	0.34±0.06^{a}	1.65±0.24^{a}
Serti	CC	1.37±0.23^{b}	1.15±0.19^{b}	0.21±0.03^{a}	1.45±0.24^{a}
	CT	1.22±0.36^{c}	1.02±0.30^{c}	0.19±0.05^{a}	1.26±0.40^{b}
	TT	1.42±0.34^{a}	1.19±0.27^{a}	0.21±0.05^{a}	1.46±0.31^{a}
Total Population	CC	1.40±0.27^{b}	1.18±0.22^{b}	0.27±0.08^{a}	1.48±0.28^{b}
	CT	1.33±0.34^{c}	1.12±0.29^{b}	0.26±0.09^{a}	1.35±0.38^{c}
	TT	1.48±0.30^{a}	1.32±0.32^{a}	0.27±0.08^{a}	1.55±0.29^{a}
κ- casein					
Arbi	AA	1.47±0.30^{a}	1.27±0.28^{a}	0.34±0.06^{a}	1.55±0.32^{a}
	AB	1.40±0.28^{a}	1.24±0.35^{a}	0.31±0.05^{a}	1.46±0.27^{a}
Serti	AA	1.35±0.30^{a}	1.13±0.24^{a}	0.21±0.04^{a}	1.40±0.31^{a}
	AB	1.35±0.33^{a}	1.15±0.30^{a}	0.21±0.05^{a}	1.42±0.35^{a}
Total population	AA	1.42±0.30^{a}	1.21±0.27^{a}	0.28±0.08^{a}	1.49±0.32^{a}
	AB	1.36±0.31^{a}	1.17±0.30^{a}	0.23±0.06^{a}	1.43±0.33^{a}

$^{a, b, c}$ values with different superscripts within the same column are significantly different (p<0.5).

statistically for the entire population. No significant relationships were found between the polymorphic forms of the β-lactoglobulin gene and the concentrations of Ca, P, Na, and K in Arbi goats.

In the case of k-casein (Table 6), the presence of the B allele in both the homozygous and heterozygous states was not associated with statistically significant differences (P > 0.05) in mineral levels in milk at the subgroup and population levels.

DISCUSSION

The results showed that Arbi goats outperformed the Serti subgroup in all physicochemical contents and macromineral concentrations studied, except for fat content, which favored Serti goats. Khaldi *et al*. (2022) reported similar results while working on the same goat subgroups reared in the western oasis of Tunisia. The current findings are in accordance with those of local Tunisian goats raised in the eastern oasis region (Ayeb *et al*., 2016), Greek breeds (Kondyli *et al*., 2012), Somali and Boer goats grazed on natural pasture (Mestawet *et al*., 2012), Nguni, Boer, and nondescript goats raised in an extensive production system (Idamokoro *et al*., 2017), Nordic and Saanen goats (Ferro *et al*., 2017), Maltese and Saanen goats (Currò *et al*., 2019) and Algeria-Arabia (Hamidi *et al*., 2020). Higher levels of milk composition traits have been cited for the Murciana-Granadina, Buren, and La Mancha breeds (Ferro *et al*., 2017) and Saanen, Camosciata dell Alpi, Murciano-Granadina, Maltese, Sarda and Sarada Primitiva goat breeds (Vacca *et al*., 2018). Differences in existing literature findings could be related to the breeds, region, lactation stage at which samples were collected, and management conditions, including environmental factors and diet (Currò *et al*., 2019).

The β-lactoglobulin and κ-casein genes were successively amplified by polymerase chain reaction. The amplified fragments were 710 and 645 bp in size for β-lactoglobulin and κ-casein genes, respectively.

β-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-lactoglobulin gene on chromosome number 3 consists of seven small exons and six introns containing 7379 nucleotides (El-Shazly *et al*., 2012). In goats, β-lactoglobulin has been allotted to chromosome 11q28 (Folch *et al*., 1994; Bawden *et al*., 1994) and to the transcription unit of 4698 bp encoding β-lactoglobulin, 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-lactoglobulin gene is similar to that of its sheep and cattle counterparts; the sizes of introns and exons are well conserved (Bawden *et al*., 1994, Cardona *et al*., 2016). In the present study, the PCR-RFLP result gives three different genotypes, each characterized by a different number and size of the fragment. The polymorphic site consists of a single nucleotide substitution (C to T) at position -60 of the goat β-lactoglobulin 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 (Yahyaoui *et al*., 2000). The frequency of the β-lactoglobulin C allele was found to be higher than that of the β-lactoglobulin T allele in Arbi, Serti, and Total population. The relatively high frequency of C allele in local goats corresponded to higher prevalence of CC genotype.

The presence of allele C at a high frequency and the predominance of CC genotypes across the oasis native goats are consistent with previous reports on goat species. β -lactoglobulin C and T alleles were identified in European goat breeds. Yahyaoui *et al*. (2000) reported that the C allele was observed frequently in Spanish breeds, including Canaria, Murciano-Granadina, Malagvena, Saanen, and Payoya with a frequency equal to 1, 0.86, 0.75, 0.73, and 0.73%, respectively. In Hungarian milky goats, the allelic frequency of the C allele was 0.88% (Veress *et al*., 2004). Working on native goats and three exotic breeds reared in Lithuanian, Baltrėnaitė *et al*., (2009) advanced a superiority of C allelic frequency compared to that of T allele with frequency ranging from 0.72 (Czech and German White goats) to 0.91 (native goats) % for C allele and from 0.087 (native goats) to 0.28% (Saanen goats) for T allele. The last authors reported the higher dominance of homozygous CC genotype compared to TT and CT genotypes with high rates in native goats (0.87%) and a lesser degree in Saanen (0.57%), Czech White (0.55%), and German White (0.56%) goats. Among different breeds studied in Egypt (Ahmed and Othman, 2009), the polymorphism within the proximal promoter region and exon 1 of β-lactoglobulin displayed three genetic variants CC, TT, and CT at different frequencies. The results obtained for the Baladi and Barki breeds corroborate our findings, while Damascus and Zaraibi breeds showed that the CT and TT genotypes were more common than the CC genotype. Similar results have been reported in the Egyptian Sahrawi breed with amplified 710-bp β-lactoglobulin fragments digested with SmaI enzyme (Ahmed and Othman, 2005).

Regarding the κ-casein polymorphism, two alleles, A and B, were found in the local goat population, resulting in two genotypes, AA and AB, with allele A and AA genotype dominating the overall population, at the subgroup level, and regardless of region. Similar results were found in Indian Surti goats (Kumar *et al*., 2009), Spanish Murciano-Granadina goats (Caravaca *et al*., 2011), 14 subpopulations of goats in Antioquia, Colombia (Atehortúa *et al*., 2012), Alpine, Saanen, and crossbred breeds (Cardona *et al*., 2015), and Colombian tropical dairy goats (Cardona *et al*., 2016).

The current study reports the association between β –lactoglobulin and κ-casein genes and milk composition traits. It was estimated that the homozygous genotypes CC and TT of β –lactoglobulin locus affected acidity averages in Serti subgroup and at the total population level but not in Arbi goats. Likewise, β –lactoglobulin variants are not associated with pH or density. Kyselová *et al*., (2019) did not describe any relationship between the β –lactoglobulin genetic polymor¬phism and the milk pH and density in Czech Fleckvieh cows. According to the findings presented in this paper, κ-casein gene variants are not associated with milk physical characteristic traits in local goats. These results are in agreement with previous findings published by Yousefi *et al*., (2013) in indigenous Iranian Zel sheep, Jawasreh*et al*., (2019) in Awassi sheep, and Kyselová *et al*., (2019) in Czech Fleckvieh cows.

Analyzing the β-lactoglobulin genotypes effects on milk chemical compositions at the level of total population, the homozygous CC and TT goats performed better than heterozygous CT goats in terms of dry matter, protein, fat, casein, and lactose contents. A similar trend was observed for the Arbi subgroup, which favored homozygous genotypes over heterozygous genotypes, except for the fat content, which was similar across the three genotypes. This is congruent with the results reported for Saudi sheep (El-Shazly *et al*., 2012), Indian goats (Kumar *et al*., 2006), and Chinese Holstein cattle (Alim *et al*., 2015).Considering Serti goats, the TT variant of the β-lactoglobulin gene had a very large positive effect on dry matter, protein, fat, and casein content compared to CC and CT genotypes. This result is consistent with some published data (Jawasreh*et al*., 2019). However, the β-lactoglobulin genotype had no effect on lactose content, which is coherent with some published reports (El-Shazly *et al*., 2012). As regards ash content, results indicated no association with β-lactoglobulin polymorphisms in total population or at subgroup level. This result complies with those of Dario *et al*., (2008) and Kawecka and Radko (2011). Many authors have reported conflicting relationships regarding β-lactoglobulin polymorphisms and milk composition (Kawecka and Radko 2011; El-Shazly *et al*., 2012).This inconsistency may be ascribed to breed differences, population size, frequency distribution of genetic variants, and structure of the analyzed data and model used for statistical anal-

ysis (Giaccone *et al*., 2000; Dario *et al*., 2008).

The present study did not confirm the significant effect of κ-casein genotypes on all milk chemical composition traits in Arbi subgroup, on dry matter, fat, lactose and ash content in Serti goats, and on lactose and ash at total population level. In studies of effect of κ-casein on chemical composition, several authors have reported no significant relationship between κ-casein polymorphism and dry matter (Bankar *et al*., 2018; Soyudal *et al*., 2018; Albarella *et al*., 2020), protein (Deb *et al*., 2014; Gras *et al*., 2016; Jawasreh *et al*., 2019), casein (Comin *et al*., 2008; Albarella *et al*., 2020), fat (Cardona *et al*., 2015; Gras *et al*., 2016; Jawasreh *et al*., 2019), lactose (Bankar *et al*., 2018; Jawasreh *et al*., 2019), or ash (Al-Shawa, *et al*., 2019; Albarella *et al*., 2020).

Our results showed a significant effect of the κ-casein variants on milk composition traits as seen in the milk of total population with respect to the milk dry matter, protein, fat, and casein content. These data indicate the superiority of the AA genotype over AB genotype for dry matter, protein, and casein means values. Similar trends were also observed for protein and casein at the level of Serti goats. Nevertheless, trends in fat content were opposite among total population and Serti subgroup, where genotype AB performs better than genotype AA. Associations between κ-casein variants and milk composition characteristics have been reported by previous literature sources in goats (Caravaca *et al*., 2009; Catota-Gómez *et al* 2017; El-Shazly *et al*., 2017), sheep (Yousefi *et al*., 2013), and cattle species (Alim *et al*., 2015; Gai *et al*., 2021). The correlations between the κ-casein gene polymorphism and the milk traits obtained by other researchers are controversial, partly due to the differences in studied breeds and/or depending on geographical origin (Hristov *et al*., 2012).

Nonsignificant associations were observed between β –lactoglobulin variants and milk calcium or potassium in Arbi goats, and between β –lactoglobulin and sodium in all studied animals. Likewise, the current study revealed a positive effect of β –lactoglobulin variants on Ca and K in Serti subgroup and at the level of total population, while a positive influence has been observed for milk phosphorus in all animals being studied.The Ca, K, and P contents of milk were estimated in the decreasing order of TT > CC > CT according to β –lactoglobulin genotypes. Many authors described significant influence of β –lactoglobulin types on major minerals of milk (Bittante *et al*., 2012; Kyselová *et al*., 2019). Devod *et al* (2000) and Kübarsepp *et al* (2005), in contrast, did not observe any significant effect of β –lactoglobulin variants on mineral contents.

No determinant impact of κ -casein genotypes was found on all studied minerals. Our findings appear to be consistent with those of Walawski *et al*., (1994) and Kyselová *et al*., (2019) who found no significant association between κ -casein genetic variants and mineral concentrations. In other studies, a positive relationship has been mentioned (Vafin *et al*., 2021).

As outlined by Gai *et al*., (2021), the structures of the main proteins in milk, including β- casein, αS1- casein, αS2- casein, κ- casein, α- lactalbumin, and β- lactoglobulin are influenced by genetic variants, as these lead to modifications of amino acid sequences. These structural differences affect milk composition and quality, as well as the isoelectric points and electric charges of the proteins, and ultimately influence the physicochemical properties of milk. Nevertheless, conflicting results have been reported in studies seeking associations between κ -casein and β –lactoglobulin phenotypes and milk composition traits. Some of the reasons for the lack of compatibility in the results may be due to differences in population size, breed of animal, frequency of genetic variants under consideration, methods of measuring production traits, lack of understanding of the relatedness of the investigated animals (Selvaggi *et al*., 2015) and most importantly the rigor of statistical analysis used to adjust for other important factors contributing to milk production such as age of animal, season, stage of lactation, health status and effects of other genetic variants (Ng-Kwai-Hang and Grosclaude, 1992).

Studies seeking the relation of the various yield and quality features of milk with the genetic variation in the milk proteins such as β-Lactoglobulin and κ -casein may lead to the identification of some molecular genetic markers as selection criteria for milk production. Nonetheless, more in-depth studies of the effect of these markers and milk production traits in local goats, involving a larger number of sampled animals and herds, are required to develop effective selection criteria for higher milk yield and better composition. Furthermore, screening the other regions of the two studied proteins, as well as investigating the interaction and combined genotype effects of these genes and in combination with other genes, could be useful in clarifying the efficiency of target genes in order to incorporate them into the characterization, preservation, and improvement strategies for autochthonous genetic resources.

CONCLUSION

The current study contributes to improving knowledge on a local goat population raised in the southwestern oasis region under a specific traditional herding system as it is focused on the investigation of genetic variants of two lactic proteins and their association with milk composition traits. The κ –casein protein had two alleles A and B, as well as two genotypes AA and AB, whereas the β - lactoglobulin protein had two alleles C and T, and three CC genotypes TT and CT, with allelic and genotypic frequencies varying by region (Jerid and Nefzawa) and subpopulation (Serti and Arbi).The genetic polymorphism of β –lactoglobulin has been linked to milk acidity, dry matter, protein, casein, fat, lactose, Ca, P, and k content, with homozygous TT and CC genotypes clearly outperforming CT genotype. The κ –casein locus had a marked influence on the dry matter, protein, casein, and fat components, favoring homozygous AA goats in all traits except fat, which distinguished heterozygous AB goats.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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