*Romanian Biotechnological Letters* *Vol. 22, No. 5, 2017*

**ORIGINAL PAPER**

# Genetic polymorphism in some milk protein genes and its impact on milk composition of Saudi Arabian goat breeds reared in Taif region

Received for publication, October 10, 2014
Accepted, January 2, 2015

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## Abstract

*The variation in Kappa-casein (κ-casein) gene and the distribution frequencies of its variants between Saudi Arabian Ardi and Syrian goat breeds were investigated. Nucleotides of 421 bp of exon 4 from both breeds were amplified and sequenced. Four nonsynonymous substitutions were recorded and three haplotypes A, B and D were found. The haplotype A occurred at the highest frequency (0.78-0.83), while the haplotype D showed the lowest rate of 0.22. The allele A was greater in fat contents in both breeds, while protein contents were higher in the allele A for Ardi and in the allele D for Syrian breed. The allele A was higher in fat contents in both breeds, while protein contents were higher in the allele A for Ardi and in the allele D for Syrian. Restriction fragment length polymorphism (RFLP) of exon 7 of β-lactoglobulin (β-Lg) revealed that three genotypes (AA, AB, and BB) were detected in both breeds. They showed frequencies of0.14, 0.46 and 0.4 in Ardi and frequencies of 0.22, 0.17 and 0.31 in Syrian. The higher fat percentage and total solids were found in AB genotype of Ardi and AA genotype of Syrian. Protein and lactose percentages and non-fatsolid (NFS) did not show a correlation with genotype. Other casein loci are necessary to be studied to establishing haplotype effects on milk production traits.*

**Keywords:** β-lactoglobulin, κ-casein, polymorphism, goat, Saudi Arabia

## 1. Introduction

Goats are the first domesticated animals for meat and milk production. Among all foods, milk represents the most complete and balanced diet in nutritional contents (1). The presence of similar percentages of protein, fat, and carbohydrates gives the milk a characteristic indestructibility over any period (2, 3). Compared to cow, goat milk is higher in monounsaturated, polyunsaturated fatty acids and medium chain triglycerides which are all known to play a major role for human health especially in cardiovascular conditions (4). Despitethe superiority of goat milk, both cattle and sheep have had the interest of the researchers. Genetic polymorphism of milk proteins has received considerable interests in breeding because of their relationships with production traits, milk composition and milk quality (5). The milk protein loci are highly polymorphic in nature (6). These loci might be used as genetic markers for the animal selection in breeding programs (7). The relationship between genetic polymorphism and milk quality and quantity in casein genes has stimulated the interest to use this polymorphism in molecular marker-assisted selection (MAS) to improve milk production in farm animals (8). Milk proteins genetic polymorphism was reported for the first time by Aschaffenburg and Drewry (9) and since then, many studies

have been conductedin this respect. This has led to an extensive investigation of genetic polymorphism in the milk proteins including β-Lg and therefore, several variants were identified (10, 11).β-Lg is the major component in whey of ruminants with two main variants detected and described as β-Lg A and β-Lg B and one rare variantβ-Lg C (12, 13). Three genetic variants of β-Lg occur in sheep milk designated by the letters A (fast), B (intermediate) and C (slow) (13). κ-casein plays an important role in the formation, stabilization, and aggregation of the casein micelles and thus it alters the manufacturing properties and digestibility of milk (14). The casein proteins are encoded by a locus that comprises four casein genes which are three evolutionary related calcium-sensitive casein encoding genes (αs1, αs2, and β) and one function-related κ-casein gene (15).The relationship between κ-casein polymorphism and milk quantity and quality has been studied in different breeds including Indian (16), African (17), Camosciate, Srisa, Orobica, Verazsca(18), German (19) and Egyptian goat breeds (20). Some allelic variants have been identified which were primarily classified into two groups. Group B (D, E, K, and M)has been shown to have a positive effect on milk yield and technological properties. Group A alleles have a less positive influence on milk composition (17). Many researchers believe that the κ-casein B variant is associated with higher fat, protein, and casein and has a significant impact on cheese making properties and superior rennet coagulation properties in comparison to AA or AB variants (21). The current study aimed to analyze the genetic polymorphism of both exon 4 of κ-casein gene and exon 7 of the β-Lg gene in Ardi and Syrian goat breeds reared in Taif region of Saudi Arabia. It also aimed to determine the distribution frequencies of their variants and to study the influence of their polymorphism on milk composition.

## 2. Materials and methods

### Sample collection and genomic DNA extraction

Blood and milk samples were collected from thirty Ardi and eighteen Syrian ewes living in natural habitats in Taif region of Saudi Arabia. Blood samples were drawn into potassium EDTA evacuated blood collection tubes, transported to the lab in ice box and then preserved at -20 °C till further use. Milk samples were collected from lactating ewes (between one month and three months lactating period) into 15 ml Falcon tube and preserved at -80 °C until analysis. Genomic DNA was extracted from whole blood using AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biosciences 33210 Central Avenue, Union City, CA 94587 USA) according to the manufacturer's instructions. DNA concentration and purity were examined using UV spectrophotometer at 260nm and280 nm.

### Polymerase chain reaction (PCR)

Amplification of exon 4 of κ-casein gene and exon 7regionsof the β-Lg gene was performed usingspecific primers (Table 1).

Table 1. Primers used in PCR experiments

| Gene | Product size | Annealing | Direction | Sequence |
|---|---|---|---|---|
| κ-casein | 458 bp | 59 ºC | sense | TATGTGCTGAGTAGGTACC |
| | | | antisense | TTGTCCTCTTTGATGTCTCC |
| β-Lg | 780 bp | 58 ºC | sense | AGGAAGTGGGTACCTAAGGG |
| | | | antisense | ATACCGACAGTAGTGGCTGG |

PCR was conducted in a final volume of 50 µl consisting of 1µl DNA, 1µl of 10 *p*M of each primerand25µl PCR master mix (Promega Corporation, Madison, WI, USA) the volume was brought up to 50 µl using sterilized deionized water. PCR was carried out using a PeX 0.5

thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) with the cycle sequence of 94 ºC for 5min one cycle, followed by 30 cycles each of which consisted of denaturation at 94 ºC for 1 min, annealing at the specific temperature corresponding to each primer set (Table 1) and extension at 72 ºC for 1min with an additional cycle for a finalextension at 72 ºC for 5min. PCR products were electrophorized on 1.5% agarose (Bio Basic, Konrad Cres, Markham, ON, Canada), gel stained with ethidium bromide in TAE (Tris-acetate-EDTA) buffer (Sigma-Aldrich, St. Louis, MO, USA). PCR products were visualized under UV light and photographed using gel documentation system(UVP, Upland, CA, USA). Then, PCR product was purified using FavorPrep PCR Clean-Up Mini Kit according to the manufacturer's instructions.

**RFLP analysis**

A total volume of 4 µl of each purified PCR product was digested overnight at 37°C with 10 U of (Cfr42I) *Sac*II and (BsuRI) *Hae*III (Fisher Scientific Pittsburgh PA, USA) endonuclease for β-Lg and κ- casein, respectively. Digested products were analyzed by electrophoresis on 2% agarose gel and then stained with 1% ethidium bromide and photographed by gel documentation system.

**κ-casein exon 4 sequence analysis**

Purified PCR products forκ-casein were sequenced on an ABI PRISM 3730xl sequencer (Applied BioSystems) and BigDyeTM Terminator Sequencing Kits with AmpliTaq-DNA polymerase (FS enzyme) (Applied Biosystems)following the protocols supplied by the manufacturer. After reading the targeted genes, the nucleotide sequences have been treated with different software programs (DNASIS, MacClade, PAUP) that enabled us to detect genetic relatedness between different samples.

**Milk chemical composition analysis**

Milk composition including total protein, total fat, lactose, total solids and non-fat solid(NFS) contents were determined using Milko-scan 130 series system maintained at International Live Stock Management Training Center (ILMTC) which follows Animal Production Research Institute (APRI) Sakha, Kafr El-Sheikh Provence, Egypt.

**Statistical analysis**

Statistical analysis was performed using analysis of variance (ANOVA) and Duncan's least-significant difference test, by SPSS package v.13 program with $p< 0.05$ regarded as statistically significant.

## 3. Results

**Variation in κ-casein gene**

A fragment of exon 4 of κ-casein gene (CSN3) with 458 nucleotides was amplified in this study and the restriction enzyme *Hae*III produced two fragments of 229 bp for each (Figure1).

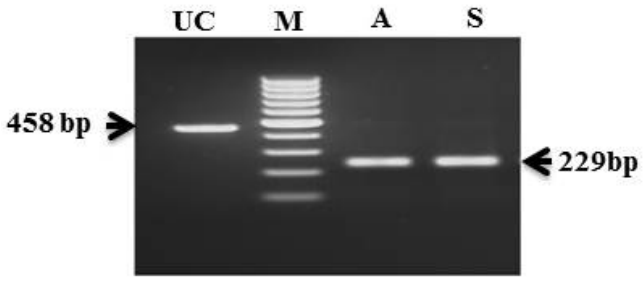


**Figure 1.** Electrophoretic profile of PCR products for exon 4 of κ-casein gene of Ardi and Syrian goat breeds digested with *HaeIII* restriction enzyme. M indicates 100 bp DNA size marker, UC is uncut PCR product 458 bp. Other lanes are PCR products from different individuals from Ardi (A) and Syrian (S) breeds.

This sequenced fragment was analyzed for 30 Ardi and 18 Syrian individuals to highlight their nucleotides and amino acid variations. These data were deposited in DDBJ/EMBL Genbank database with their accession numbers (AB935556- AB935583 for Ardi and AB935584 - AB935601 for Syrian). Point mutations for five polymorphic sites were identified (Table 2) which were transitions between T and C or between Aand G. Analysis of the translated amino acid showed that all substitutions are conservative and only one site was a synonymous corresponding to an amino acid residue of glutamine at the position 247. The other four mutations were non-synonymous resulting in the substitution of glutamine with arginine (at a position 245), valine to isoleucine (309), isoleucine to valine (471) and serine to proline (591) (Table 3).

Three haplotypes were detected with different alleles with only one haplotype occurred at a rather high frequency (Table 3). The prevalent κ-casein variant was CSN3*A with frequencies ranging from 0.78 (Syrian)to 0.83 (Ardi). The second common allele was CSN3*D which was found in only one sample of Ardi breed and constituted approximately one fourth of the Syrian breed (Table 2).

Table 2. κ-casein gene haplotypes for Saudi Arabian Ardi and Syrian goat breeds. Nucleotide positions with their translated amino acids are compared to that of the Genbank (accession number X60763).

| Nucleotide position | Amino acid position | Genotypes | | | |
|---|---|---|---|---|---|
| | | A | B | D | D |
| | | Ardi & Syrian | Ardi | | Syrian |
| 245 | 43 | T (Gln) | C (Arg) | | |
| 247 | 44 | A (Gln) | G (Gln) | | |
| 309 | 65 | G (Val) | A (Ile) | | |
| 471 | 119 | A (Ile) | | G (Val) | G (Val) |
| 591 | 159 | T (Ser) | C (Pro) | | |

The relationship between κ-casein haplotype and milk chemical composition was investigated. The results showed that in Syrian breed, the haplotype A was higher in fat and total solids while in Ardi breed the genotype A was higher in protein and fat contents (Table 3).

Table 3. Haplotype frequencies of the κ- casein locus in the two goat breeds and their milk chemical composition. Star indicates the significant difference between alleles of the same breed.SNF refers to solid non fat content.

| Breed | Allele | Frequency | Protein % | Fat % | Lactose % | Total Solids % | SNF % |
|---|---|---|---|---|---|---|---|
| Ardi | A | 0.83 | 3.53± 0.09* | 3.98 ± 0.15* | 3.75 ± 0.12 | 11.8 ± 0.22 | 7.85 ± 0.12 |
| | B | 0.17 | 3.131 ± 037 | 3.45 ± 022 | 3.47 ± 0.23 | 11.0 ±0 .31 | 7.56 ± 0.43 |
| Syrian | A | 0.78 | 3.76 ± 0.25 | 4.90 ± 0.35* | 4.09 ± 0.1* | 13.13 ± 0.6 | 8.18 ± 0.29 |
| | D | 0.22 | 4.45 ± 0.26* | 4.51 ± 0.52 | 3.14 ± 0.59 | 12.87 ± 1.8 | 8.19 ± 0.60 |

**Variation in exon 7 of β-Lg gene**

PCR productwith682 bp of β-Lg gene exon 7was detected. Digestion of the PCR product using *Sac*II endonuclease enzyme revealed three genotypes (Figure2).

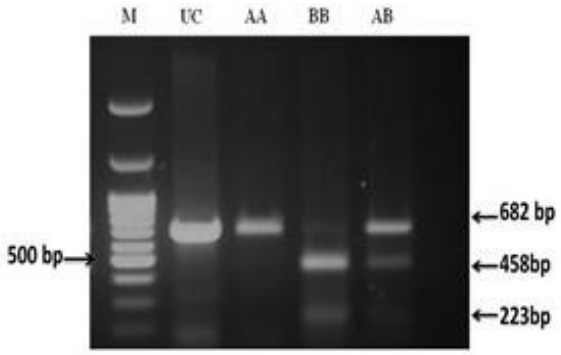


**Figure 2.** Electrophoretic profile of PCR products from exon 7 of β-Lg gene of Ardi and Syrian goat breeds. M indicates 100 bp DNA size marker and other lanes are PCR products from different individuals from Ardi and Syrian breeds.

These genotypes were AA (one fragment 682 bp), AB(three fragments 682, 458 and 223 bp) and BB (two fragments 458 and 223 bp) with genotypic frequenciesof 0.14, 0.46 and 0.40 in Ardi, whilefor Syrian breed,their genotypic frequencies were 0.22, 0.17 and 0.61, respectively (Table 4). The relationship between genotype and milk chemical composition was investigated. The results showed that the genotype AB showed high fat and total solids contents in Ardi, while in Syrian the genotype AA was higher in fat and total solids contents. Other parameters including protein, lactose and sold non-fat showed insignificant differences (Table 4).

Table 4. Genotypic frequencies for the β-Lg locus in the two goat breeds and their milk chemical composition. Star indicates the significant difference between different genotypes within the same breed. SNF refers to solid non fat content.

| | Genotype | Genotypic distribution | Protein % | Fat % | Lactose % | Total Solids % | SNF % |
|---|---|---|---|---|---|---|---|
| Ardi | AA | 0.14 | 3.98±0.46 | 4.10±0.09 | 3.29±0.68 | 11.86±0.46 | 7.87±0.26 |
| | AB | 0.46 | 3.48±0.12 | 4.31±0.2* | 3.95±0.12 | 12.30±0.28* | 7.90±0.17 |
| | BB | 0.40 | 3.37±0.14 | 3.44±0.19 | 3.60±0.09 | 11.02±0.25 | 7.91±0.23 |
| Syrian | AA | 0.22 | 4.23±0.72 | 6.1±0.76* | 3.87±0.19 | 14.83±1.40* | 8.72± 0.6 |
| | AB | 0.17 | 3.62±0.81 | 4.40±0.20 | 3.52±0.8 | 12.13±0.40 | 7.74±0.25 |
| | BB | 0.61 | 4.33±0.34 | 5.17±0.20 | 3.98±0.18 | 14.09±0.54 | 8.90 ±0.4 |

## 4. Discussion

Genetic polymorphism in milk proteins was firstly reported by Aschaffenburg and Drewry (9). Since then many studies have been performed since genetic polymorphisms play asignificant role as genetic markers in the animal breeding programs. The association of β-Lg polymorphism with milk yield and composition has been reported in cows, sheep and Indian goats (10, 22, 23). However, this relationship has not been clarified yet in Saudi Arabian goat breeds. In the present study, PCR digested with SacII revealed three genotypes; AA (0.14, 0.22), BB (0.46, 0.17) and AB (0.40, 0.31) for Ardi and Syrian breeds, respectively. The physicochemical characteristics of milk are important for the efficient development of milk industry and marketing of its products. Although the effect of milk protein polymorphism on milk production traits has been investigated since past decades, results are still conflicting (24). Therefore, β-Lg locus has been extensively studied as one of the genes that may affect the economically important traits. Some studies observed that β-Lg polymorphism significantly affects milk yield (25), milk fat composition and protein content (26). However,

other studies failed to detect any effect of the genetic polymorphism on milk production traits (27). In the present study, the relationship between β-Lg genotype and milk composition has been investigated, and the data showed breed-dependent results. In Ardi, the genotype AB showed high fat and total solids contents while in Syrian, the genotype AA was higher in fat and total solids contents.

The κ-casein gene has been reported as a highly polymorphic gene (28-30). The association of genetic polymorphism with milk production and composition has stimulated the interest in using genetic polymorphism of casein genes in molecular marker-assisted selection (MAS) to improve milk production in farm animals (8). However, many studies on goat CSN3 showed that the gene is highly polymorphic (14, 19, 28-30).

Sequence variations of exon 4 of κ-casein gene in the present study revealed five SNPs which had been previously reportedin some East African goat populations (17). Similar to the present study, Yahyaoui et al. (29) identified the polymorphism in Spanish and French breeds in positions 245, 309, 471 and 591. The polymorphism in the position 247 was first identified by Caroli et al. (19) in Italian goat breeds. These SNPs have also been described in various goat populations (14, 30). Kiplagat et al. (17) suggested that the high similarity in polymorphic sites amongst most of the global populations is most probably due to the similarity in evolutionary processes undergone for them. It is very interesting to elucidate a relationship between any of these polymorphisms and κ-casein protein structure and function which can be used as markers for milk production traits. Most of the genotypes at polymorphic sites were heterozygous. Only the polymorphic site 471 was homozygous for genotype AA in approximately 90% of the samples analyzed from both breeds. This result was in agreement with the observations of Kiplagat et al. (17) and Mercier et al. (28). The studies mentioned above, together with the current study, postulated isoleucine to be the predominant amino acid in the corresponding amino acid sequence. The relationship between casein allele and milk chemical composition showed that the allele A was higher in fat contents in both breeds, while protein contents were higher in the allele A for Ardi and in the allele D for Syrian.

## 5. Conclusion

In conclusion, the present study showed types and distribution of κ-casein alleles and β-Lg genotypes in both Ardi and Syrian goat breeds reared in Taif region. In addition,it clarified the relationship between alleles and milk composition. The relationship between alleles and milk composition might be breed-dependent. Further studies on other casein loci are necessary to establish associations of all the casein mutations and the effects of the haplotypes to milk production traits.

## Acknowledgement

This work has been done under the financial support of Taif University of Kingdom of Saudi Arabia (Project number 1/434/2553).

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