ORIGINAL ARTICLE JGEB Vol. 7, No. 2, 2009
ISSN:1687-157X

Genotyping Analysis of Milk Protein Genes in Different Goat Breeds Reared in Egypt

Sahar Ahmed and Othman E. Othman

Cell Biology Department, National Research Center, Dokki, Egypt

ABSTRACT

The genes that encode the major protein and whey protein of milk are candidate genes in molecular marker assisted selection to improve the milk productivity in farm animals. Our study concerned with genotyping analysis of four milk genes, CSN1S1, CSN1S2, KCN and β-LG in four goat breed reared in Egypt; Baladi, Barki, Damascus and Zaraibi. Our results revealed that the CSN1S1 allelic variants in tested breeds showed five different genotypes, three of them were homozygous (A/A (4.5%), B/B (6.8%) and D/D (2.3%)) and the other two were heterozygous (A/C (40.9%) and B/D (45.5%)). For CSN1S2 gene, the Egyptian goat breeds carry the A, B and F alleles, while the C, D, E and 0 alleles were not present. The frequency of homozygous genotypes AA, BB were 28.9% and 26.7%, respectively, while heterozygous genotypes AB and AF were 40% and 4.4%, respectively. The k-CN results illustrated that among different Egyptian breeds, B allele was the most common allele in breeds with maximum frequency in Zaraibi breed (90%), where the allele C with low frequency in Barki (9.1%) and Zaraibi (8.3%) breed. Allele A was displayed in different frequencies ranged from 45.5% (Barki) to 10% (Zaraibi). Genetic polymorphism of β-LG exon 7 showed two genetic variants S_1S_2 genotype with 45.5% and S_2S_2 with 54.5%. The homozygous S_1S_1 genotype was not displayed in all tested animals. Among different breeds, the polymorphism within the proximal promoter region and exon 1 of β-LG displayed three genetic variants CC, TT and CT at different frequencies with the exception of CT which was not displayed in Zaraibi breed. In conclusion, genetic analysis of the goat milk protein genes is a valuable tool for selecting the favorable genotype of milk genes with the highest yield of milk.

KeyWords: Goat, polymorphism, milk genes, PCR-RFLP.

Corresponding Author: Othman E. Othman
E-mail: othmanmah@yahoo.com

Journal of Genetic Engineering and Biotechnology, 2009, 7(2): 33-39

INTRODUCTION

The molecular analysis of goat genome is currently carried out using specific goat sequences as well as heterologous ones isolated from cattle and sheep *(Vaiman et al., 1996)*. Deoxyribonucleic acid (DNA) based markers have a number of favorable characteristics which represent ideal tool for studying the genetic quantitative traits (QTLs) *(Ajmone-Marsan et al., 2001)*. The genetic markers applied to animal breeding and production is focused mainly on analysis of genes with economically important QTLs. The genes that encode the major protein and whey protein of milk are candidate genes of milk trait. The association of genetic polymorphism with milk production and composition has stimulated interest in using genetic polymorphism of milk protein genes in molecular marker assisted selection (MAS) to improve milk productivity in farm animals *(Ng-Kwai-Hang, 1998)*.

The analysis of Ca-sensitive caseins (CN) variation in the domesticated goat is quite complex because a large number of mutations involved the 4 coding genes *(Rando et al., 2000 and Caroli et al., 2006)*, which are tightly linked in the CN cluster *(Threadgill and Womack, 1990 and Rijnkes, 2002)*. The three Calcium-sensitive caseins αS1-CN, αS2-CN and β-CN are coded by the CSN1S1, CSN1S2 and CSN2 genes, respectively, whereas Kappa-CN is coded by the CSN3 gene. Deep relationships between the large genetic variation and functional and biological properties affecting milk quality, composition and technological characteristics have been found mainly in goat CSN1S1 *(Clark and Sherbon, 2000 and Serradilla, 2002)* which is characterized by high quantitative and qualitative variation. In addition, the CSN1S2 and CSN2 genes of the cluster have been associated with differences in the expression level of specific protein *(Caroli et al., 2006)*.

Kappa casein (K-CN) is the milk protein essential for the micelle formation and stabilization and influences the manufacturing properties of milk. It differs from other caseins in its solubility over a broad range of calcium ion concentrations and contains a hydrophilic C-terminal region *(Mercier et al., 1973)*.

Beta-lactoglobulin (β-LG) is the major whey protein in the milk of ruminants and several non-ruminant species *(Perez and Calvo, 1995)*. Schaar, et al. *(1985)* reported that milk protein genotype has a significant influence on cheese making with β-LG genotype; the effect is expressed mainly in the behavior of milk during renneting.

In Egypt, there are four main goat breeds; Baladi, Barki, Damascus and Zaraibi. Baladi breed is the most dominant indigenous Egyptian goat breeds. It is found in the Delta and the Nile Valley and its animals are black; they may be white, red or gray. The Barki breed- it is also named Sahrawi- is found in the Northwestern Coastal desert of Egypt and its animals are mainly black with or without white spots on the head and body. Zaraibi breed- it is also named Nubian or Egyptian Nubian- is found in the North East of the Nile Delta and its animals are cream, red, black or brown *(Galal et al., 2005)*. Damascus breed is not indigenous Egyptian breed; it is raised in the regions of Syria and Lebanon. It is used for crossbreeding with indigenous goat breeds for their genetic improvement primarily for milk production *(Agha et al., 2008)*. Its animals are of the Nubian type and they are red or brown in color and they can be either horned or polled.

This study was aimed to analyze the genotyping of the four milk protein genes CSN1S1, CSN1S2, KCN and β-LG in four goat breeds reared in Egypt; Baladi, Barki, Damascus and Zaraibi; Using PCR-RFLP technique.

MATERIALS AND METHODS

Animals:

The goats undertaken in this study were selected from distant experimental stations belonging to Animal Production Research Institute, Agricultural Research Center, Desert Research Center, Ministry of Agriculture and Agriculture Faculties; where random-mating strategies are employed. To minimize the likelihood of any close genetic relationships, the number of the breed samples from each station was restricted deliberately. Forty four animals that belonging to the four goat breeds; Baladi (11 goats), Barki (11 goats), Damascus (12 goats) and Zaraibi (10 goats); were undertaken in this study.

Genomic DNA extraction:

Genomic DNA was extracted from whole blood of the goat animals by phenol-chloroform method *(John et al., 1991)*. Briefly, Ten ml of blood were taken on EDTA and mixed with 25 ml cold sucrose-triton and the volume was completed to 50 ml by autoclaved double distilled water. The solution was mixed carefully and the nuclear DNA pellet was obtained by spin and discarding the supernatant. The nuclear DNA pellet was suspended at 37°C in lysis buffer, sodium dodecyl sulfate (SDS) and proteinase K. Nucleic acids were extracted once with phenol, saturated with TE buffer, followed by extraction with phenol-chloroform-isoamyl alcohol (25:24:1). This was followed by extraction with chloroform-isoamyl alcohol (24:1). To the final aqueous phase, Na acetate and cold 95 % ethanol were added. The tubes were agitated gently till a fluffy white ball of DNA was formed. The DNA was picked up and washed in 70 % ethanol. The DNA was dissolved in 1X TE buffer and diluted to a concentration of 50 ng/μl, which is suitable for PCR.

Polymerase chain reaction (PCR):

A PCR cocktail consists of 1.0 μM upper and lower primers specific for each studied gene (Table 1) and 0.2 mM dNTPs, 10 mM Tris (pH 9), 50 mM KCl, 1.5 mM MgCl2, 0.01 % gelatin (w/v), 0.1 % Triton X-100 and 1.25 units of Taq polymerase was used. The cocktail was aliquot into tubes with 100 ng DNA of goat. The reaction ran in a Coy Temp Cycler II (Coy Corporation, MI, USA). The reaction was cycled for 1 min at 94°C, 2 min at an optimized annealing temperature that is determined for each primer (Table 1) and 2 min at 72°C for 30 cycles.

RFLP and agarose gel electrophoresis:

Twenty μl of PCR product for each primer were digested with 10 units of specific restriction enzyme (Table 1) in a final reaction volume 25 μl. The reaction mixture was incubated at 37°C in water bath over night. After restriction digestion, the restricted fragments were analyzed by electrophoresis on 2.5% agarose/1X TBE gel stained with ethidium bromide. The 100 bp ladder was used as molecular size marker. The bands were visualized under UV light and photographed with yellow filter on black and white film.

Table 1: The information and DNA sequences of used primers

Gene	Primer no.	Allele detected	Sequence 5' ---------- 3'	Anneal temp. (°C)	PCR product size (bp)	Restriction enzyme	References
CSN1S1	Primer 1	A, B, C and D	TTC TAA AAG TCT CAG AGG CAG GGG TTG ATA GCC TTG TAT GT	60	223	*Xmn*I	Ramunno, et al. (2000)
CSN1S2	Primer 2	A and B	GCC ATT CAT CCC AGA AAG CTC TTC ATT TGC GTT CCT TA	54	1300	*Mse*I	Cosenza et al. (1998)
	Primer 3	A, B and C	AAT TAA CTG CTT CTA CCT GG CTC AGA AAG ATT AGG GAA AG	54	3700	*Pst*I	Ramunno, et al. (1999)
	Primer 4	0 and D	GAC ACA TAG AGA AGA TTC CGT TGG GAC ATT TTA TCT	51	301	*Nco*I	Ramunno et al. (2001)
	Primer 5	E	TTT AGG AAG CGA GGA CCA AGT A CTG AAA CTG TAG AAG ATA GAT T	56	232	*Nla*III	Lagonigro, et al. (2001)
	Primer 6	F	TCT CTT GCC ATC AAA ACA TGG TCT TTA TTC CTC TCT	54	310	*Alw*26I	Ramunno, et al. (2001)
CSN3	Primer 7	A or B and C	TGT GCT GAG TAG GTA TCC TAG TTA TGG GCG TTG TCC TCT TTG ATG TCT CCT TAG	63	459	*Alw*44I	Yahyaoui, et al. (2001)
	Primer 8	A and E	GCG TTG TCC TCT TTG ATG TCT CCT TAG TCC CAA TGT TGT ACT TTC TTA ACA TC	63	645	*Hae*III	Yahyaoui, et al. (2003)
β-LG	Primer 9	S_1 and S_2	CGG GAG CCT TGG CCC TCT GG CCT TTG TCG AGT TTG GGT GT	65	426	*Sac*II	Pena, et al. (2000)
	Primer 10	C and T	GTC ACT TTC CCG TCC TG GG GGC CTT TCA TGG TCT GGG TGA CG	63	710	*Sma*I	Yahyaoui, et al. (2000)

RESULS

Genetic polymorphism of CSN1S1:

The PCR products of primer 1 (223-bp) digested by restriction enzyme *Xmn*I allowed to identify five genotypes. Three of them were homozygous and the other two were heterozygous. The A/A genotype gave a fragment at 150-bp, B/B genotype at 161-bp, D/D genotype at 223-bp (Figure 1). The presence of A/C illustrated by two fragments at 150- and 212- bp and B/D by two fragments at 161- and 223-bp (Figure 2).

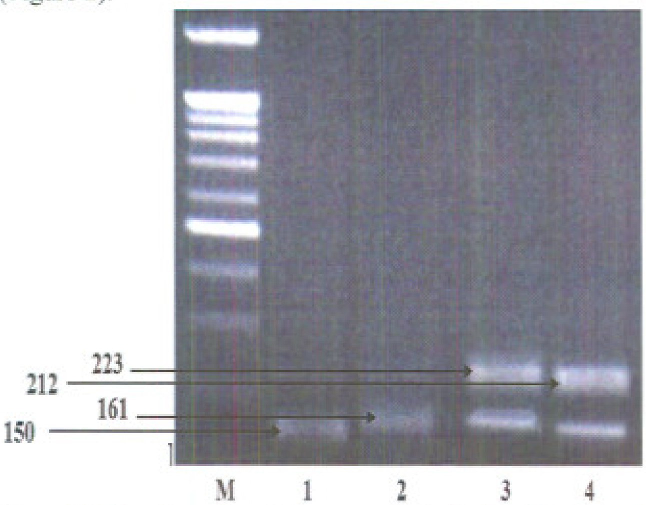

Figure 1: The electrophoretic pattern obtained after digestion of PCR amplified goat CSN1S1 products with *Xmn*I.
M: 100-bp ladder marker.
Lane 1: A/A homozygous genotype.
Lane 2: B/B homozygous genotype.
Lane 3: B/D heterozygous genotype.
Lane 4: A/C heterozygous genotype.

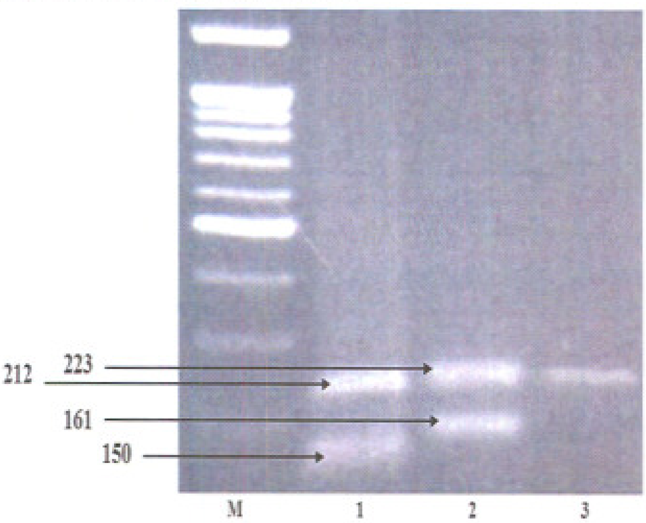

Figure 2: The electrophoretic pattern obtained after digestion of PCR amplified goat CSN1S1 products with *Xmn*I.
M: 100-bp ladder marker.
Lane 1: A/C heterozygous genotype.
Lane 2: B/D heterozygous genotype.
Lane 3: D/D homozygous genotype.

Among different breeds (Table 2), the results of genetic variants showed that the Damascus breed had five different genotypes A/A, B/B, D/D, A/C, B/D, the Baladi breed had A/C genotype with high frequency 90.9%, while the Barki breed carried two different genotypes with different frequencies. In Zaraibi breed, the genotype B/D was the most common by 80%.

Table 2: Genotype frequencies of CSN1S1 among four tested goat breeds

Tested breed	No. of animals	Allele frequencies (%)				
		A/A	B/B	D/D	A/C	B/D
Baladi	11	-----	-----	-----	90.9	9.1
Barki	11	-----	-----	-----	27.3	72.7
Damascus	12	16.7	16.7	8.3	33.3	25.0
Zaraibi	10	-----	10.0	-----	10.0	80.0
Total	44	4.5	6.8	2.3	40.9	45.5

Genetic polymorphism of CSN1S2:

A and B allele detection:

The PCR products of primer 2 (1.3-kb) digested by restriction enzyme *Mse*I showed a specific fragment of about 300-bp for allele A, while *Mse*I digestion detected allele B by giving a specific fragment of about 400-bp. In addition to these specific fragments by which we can differentiate between A and B alleles, there were two common fragments appeared at 270- and 230-bp. The AB genotype gave 4 detectable fragments at 400-, 300-, 270- and 230-bp (Figure 3). The results showed that the appearance of A allele in 33 of 44 animals (75.0%), where B allele was displayed in 30 animals (68.2%).

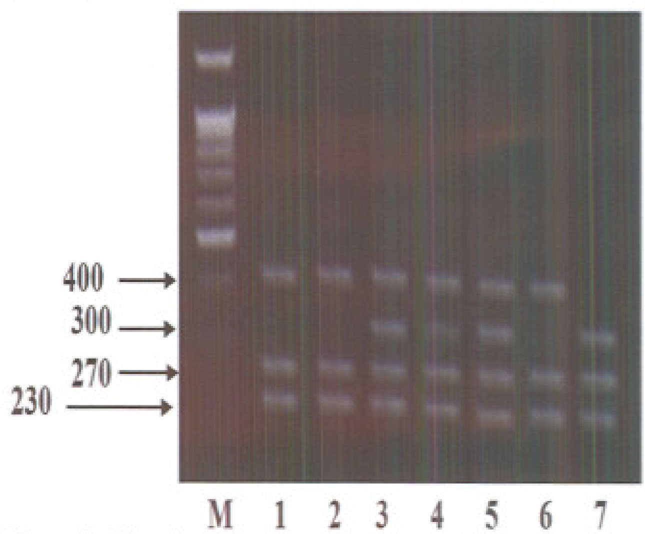

Figure 3: The electrophoretic pattern obtained after digestion of PCR amplified goat CSN1S2 products with *Mse*I.
M: 100-bp ladder marker.
Lanes 1, 2 and 6: B/B homozygous genotype.
Lane 3, 4 and 5: A/B heterozygous genotype.
Lane 7: A/A homozygous genotype.

C allele detection:

The PCR products of primer 3 (3.7 kb) digested by restriction enzyme *Pst*I showed three variant genotypes; allele A at sizes 1700-, 900- and 700-bp, allele B at sizes 1700-, 1300- and 700- bp and the genotype AB at sizes 1700-, 1300-, 900- and 700- bp. The results of the two alleles A and B were confirmed as previously mentioned using *Mse*I-RFLP. All

DNA samples extracted from four Egyptian goat breeds were not showed the fragment at 950-bp which is characterized for the C allele.

0 and D allele detection:

The PCR products of primer 4 (301-bp) digested by restriction enzyme *Nco*I presented two fragments at 168- and 133-bp in all DNA samples. The fragments of 301-bp (specific for 0 allele) and 62-bp (specific for D allele) were not displayed in all samples. This results indicated to the absence of 0 and D alleles in all tested goat animals.

E allele detection:

The PCR products of primer 5 (232-bp) digested by restriction enzyme *Nla*III allowed to identify two fragments at 142- and 90-bp in all DNA samples which revealed that the absence of the E allele, where the fragment of 232-bp (specific for E allele) was not displayed in all samples.

F allele detection:

The PCR products of primer 6 (310-bp) digested by restriction enzyme *Alw*26I showed that all DNA samples (except two samples) gave two fragments at 179- and 131-bp revealed that the absence of the F allele in the most tested animals, where undigested fragment at 310-bp (specific for F allele) was not displayed in 42 samples. Only two samples showed 3 fragments after digestion at 310-, 179- and 131-bp and revealed that these two animals have genotype F/N (Figure 4), where N is any other allele of this locus. According to the results of *Mse*I-RFLP (for detection of A and B alleles), these two animals have a genotype F/A due to the appearance of digested fragments characteristic for A allele in these two DNA samples.

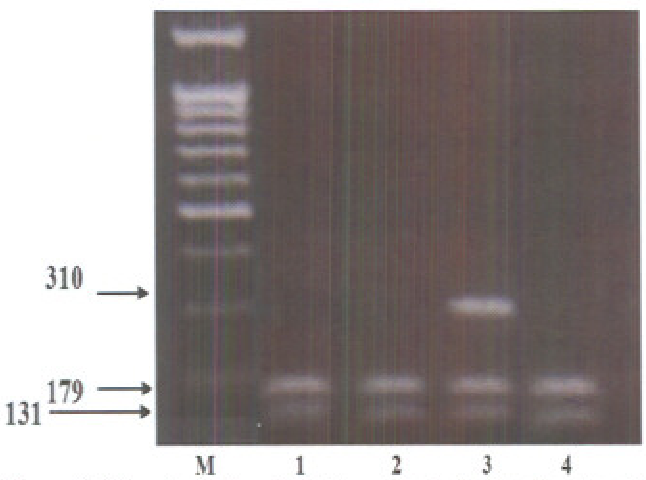

Figure 4: The electrophoretic pattern obtained after digestion of PCR amplified goat CSN1S2 products with *Alw*26I.
M: 100-bp ladder marker.
Lanes 1, 2 and 4: N/N genotype, where N= A, B, C, D, E or 0 allele.
Lane 3: F/N genotype, where N= A, B, C, D, E or 0 allele.

Genetic polymorphism of CSN3:

A or B and C allele detection:

The PCR products of primer 7 (459-bp) digested by restriction enzyme *Alw*44I produced two restriction fragments at 381- and 78-bp for allele C, while the PCR products for A and B alleles remained intact (Figure 5).

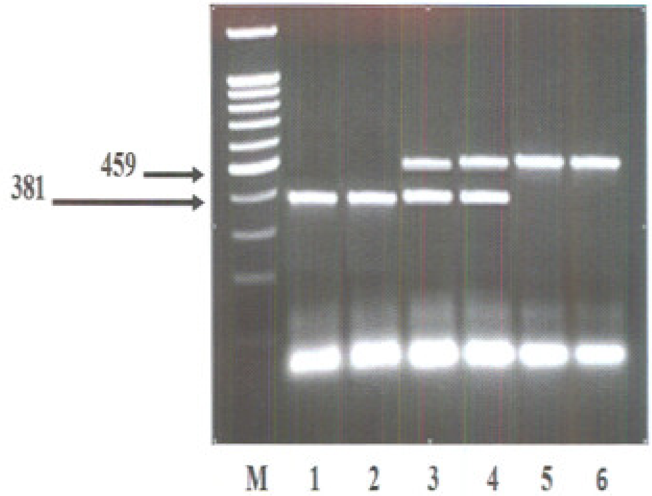

Figure 5: The electrophoretic pattern obtained after digestion of PCR amplified goat CSN3 products with *Alw*44I.
M: 100-bp ladder marker.
Lanes 1 and 2: C/C homozygous genotype.
Lanes 3 and 4: A or B/C genotype.
Lanes 5 and 6: A or B/A or B genotype.

A and E allele detection:

The PCR products of primer 8 (645-bp) digested by restriction enzyme *Hae*III produced two fragments at 416- and 229-bp which are specific for allele A. The results revealed that the absence of allele E in all tested animals, where the restriction fragments at 366-, 229- and 50- bp (specific for allele E) was not displayed in all samples. Allele B was detected by combining the results of digestion with *Alw*44I and *Hae*III endonucleases.

Among different breeds, we found that the allele B is the most common allele in the four tested breeds and appeared with the maximum frequency in Zaraibi breed (90%), where the allele C was appeared with low frequency in Barki (9.1%) and Zaraibi (8.3%) breeds. Allele A was displayed in different frequencies ranged from 45.5% (Barki) to 10% (Zaraibi) (Table 3).

Table 3: A, B and C allele frequencies of CSN3 among four tested goat breeds

Tested breed	No. of animals	Allele frequencies (%)		
		A	B	C
Baladi	11	27.3	72.7	18.2
Barki	11	45.5	54.5	9.1
Damascus	12	33.3	63.6	50.0
Zaraibi	10	10.0	90.0	8.3
Total	44	29.5	68.2	22.7

Genetic polymorphism of β-lactoglobulin:

S_1S_1, S_1S_2 and S_2S_2 allele detection:

The PCR products of primer 9 (426-bp) digested by restriction enzyme *Sac*II produced two genetic variants, S_1S_2 genotype gave three fragments at 426-, 349- and 77- bp and S_2S_2 gave one undigested fragment at 426-bp (Figure 6). The homozygous S_1S_1 genotype was not displayed in all tested animals. (Table 4) summarized the allele frequencies in the 44 tested animals.

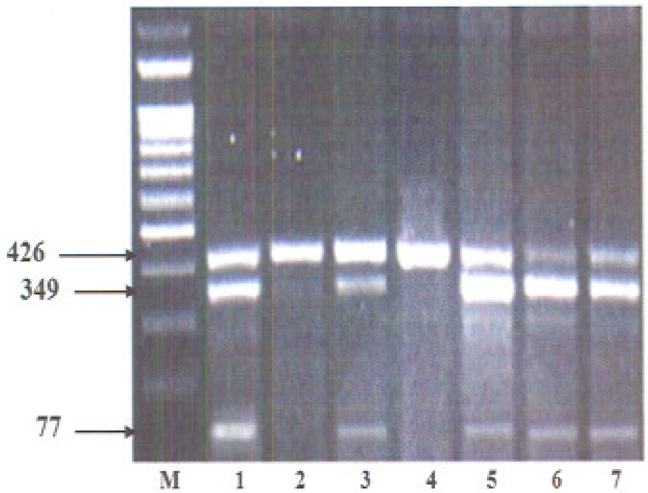

Figure 6: The electrophoretic pattern obtained after digestion of PCR amplified goat β-LG products with *Sac*II.
M: 100-bp ladder marker.
Lanes 1, 3, 5, 6 and 7: S_1S_2 heterozygous genotype.
Lane 2 and 4: S_2S_2 homozygous genotype.

Table 4: Genotype frequencies of S_2S_2 and S_1S_2 of β-LG among four goat breeds.

Tested breed	No. of animals	Genotype frequencies (%)	
		S_2S_2	S_1S_2
Baladi	11	54.5	45.5
Barki	11	------	100
Damascus	12	91.7	8.3
Zaraibi	10	70	30
Total	44	54.5	45.5

TT, TC and CC allele detection:
The PCR products of primer 10 (710-bp) digested by restriction enzyme *Sma*I showed two fragments for allele C at 472- and 181- bp, while allele T gave two fragments at 472- and 231-bp. The CT heterozygous genotype showed three digested fragments at 472-, 231- and 181-bp (Figure 7). (Table 5) summarized the allelic frequencies in different breeds.

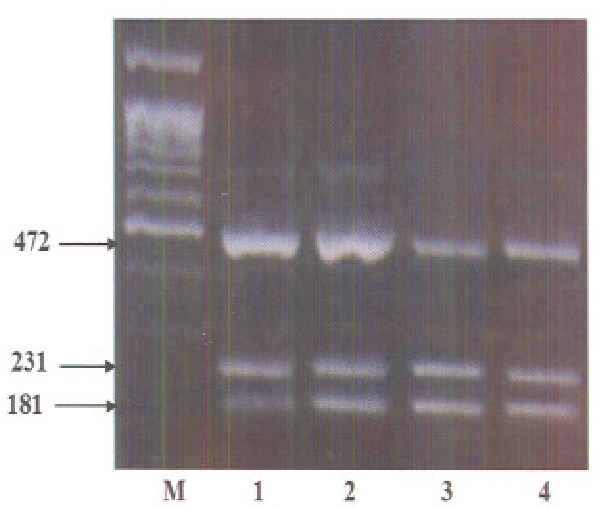

Figure 7: The electrophoretic pattern obtained after digestion of PCR amplified goat β-LG products with *Sma*I.
M: 100-bp ladder marker.
Lanes 1 – 4: CT heterozygous genotype.

Table 5: Genotype frequencies of CC, TT and CT of β-LG among four goat breeds.

Tested breed	No. of animals	Genotype frequencies (%)		
		CC	TT	CT
Baladi	11	54.5	36.4	9.1
Barki	11	72.7	18.2	9.1
Damascus	12	25.0	8.3	66.7
Zaraibi	10	20.0	80.0	-----
Total	44	43.2	34.1	22.7

DISCUSSION

In caprine breeds, CSN1S1 has 16 alleles associated with different rates of protein synthesis. On the basis of milk content of αS1-CN, the CSN1S1 variants can be group into 4 classes: Strong alleles (A, B1, B2, B3, B4, C, H, L and M), producing almost 3.5 g/l of αS1-CN each; intermediate alleles (E and D, 1.1 g/L); weak alleles (F and G, 0.45 g/l) and null alleles (01, 02 and N) apparently producing no αS1 casein *(Rando, et al. 2000 and Ramunno, et al. 2005)*. Our study revealed that the tested breeds showed low percentage of homozygous strong genotype AA (4.5%) and BB (6.8%) of milk casein used for cheese industry. The mild genotype DD recorded very low percentage (2.3%). This genotype is associated with medium level of milk protein favorable for allergic subject (nutritional purpose) especially infant diet where the goat milk with low casein is reported less allergenic than cow's milk *(Roncada, et al. 2002)*.

At least 7 alleles have been identified at CSN1S2, associated with 3 synthesis levels. The A, B *(Boulanger, et al. 1984)*, C *(Bouniol, et al. 1994)*, E *(Lagongro, et al. 2001)* and F *(Ramunno, et al. 2001)* alleles were associated with a normal αS2-CN synthesis level, whereas D and 0 were associated with lower and null synthesis levels, respectively *(Ramunno,et al. 2001)*. The results presented in this study provided that the Egyptian goat breeds carry the A, B and F alleles while the C, D, E and 0 alleles were not present. The frequencies of homozygous genotypes AA, BB were 28.9% and 26.7%, respectively, while heterozygous genotype AB and AF were 40.0% and 4.4%, respectively. Previous study by *(Ramunno, et al. 2001)* reported that the homozygous genotypes are associated with good quality of milk protein.

The results of CSN1S1and CSN1S2 in this study indicated that the quality of milk protein in Egyptian goat breeds required for milk industry is not on the level for economic issues because the developmental countries like Egypt needed to fill nutritious gap in milk and milk industry.

Recently, 16 variants of goat k-CN have been identified, involving 15 polymorphic sites in CSN3 exon 4 *(Yahyaoui, et al. 2001; Angiolillo, et al. 2002; Yahyaoui, et al. 2003; Jann, et al. 2004 and Prinzenberg, et al. 2005)*. Among different Egyptian breeds, we found that the allele B is the most common allele in the four tested breeds and appeared with the maximum frequency in Zaraibi breed (90%), where the allele C was appeared with low frequency in Barki (9.1%) and Zaraibi (8.3%) breeds. Allele A was displayed in different frequencies ranged from 45.5% (Barki) to 10% (Zaraibi) (Table 3). This could be explained due to the caprine k-CN B allele is the ancestral allele, while k-CN C allele is more divergent and the A variant shows an intermediate similarity. The difference between k-CN A and k-CN B involves only one amino acid substitution at position 119, where the valine in variants A is substituted by isoleucine in variant B. The k-CN A differs from the C variant in the following amino acid substitution: Valine for isoleucine at positions 65 and 119, alanine for valine at position 156 and serine for proline at position 159. The first amino acid substitution (position 65) occurs in the N-terminal region (caseino-macropeptide) *(Yahyaoui, et al. 2001).*

β-LG belongs to the lipocalin protein family, constituted by small secreted proteins which are characterized by their affinity to bind hydrophobic molecules. Although lipocalins have been classified primarily as transport proteins, they are implicated in several biological processes such as retinol and pheromone transport, synthesis of prostaglandins, immune response and cell homoeostasis *(Flower, 1996).* In our study, 426-bp fragment from exon 7 was amplified by PCR to detect the presence of S_1or S_2 variations. Exon 7 of goat β-LG gene comprises most of the 3' non-coding regions on the mRNA *(Folch, et al. 1994).* The difference in the mRNA stability have been reported to the cause of a reduction to one-third in the mRNA level of allele E of the goat αS1 casein gene, where E allele is considered as an intermediate allele associated with medium level of αS1 casein in milk (1.1 g/l) *(Jansa Perez, et al. 1994 and Ramunno, et al. 2000).* Therefore, the presence of S_1or S_2 alleles is necessary for the stabilization of mRNA of β-lactoglobulin. The Egyptian goat breeds have S_1S_1 and S_2S_2 genotype while S_1S_1 genotype was not displayed in tested breeds (Table 4).

Also our result showed that the polymorphism within a 710-bp PCR amplified fragment of goat β-LG gene (comprising 588-bp of proximal promoter region and 122-bp of exon 1) was detected. Several transcription factors are known to bind to recognition sequences of the goat β-LG promoter *(Watson, et al. 1991 and Folch, et al. 1994).* Among tested different breeds in this study, all genetic variants were displayed with exception of CT which was not displayed in Zaraibi breed. The genotyping with high frequencies were CC in barki (72.7%), TT in Zaraibi (80.0%) and CT in Damascus breed (66.7%) (Table 5).

In conclusion, the goat genetic polymorphism studies related to milk quality and quantity enable us to identify the favorable genotypes related to highest milk yields as well as the milk protein contents that are of most interest because of the direct relationships between milk quality composition and technological characteristic.

REFERENCES

Agha, S. H., Pilla, F., Galal, S., et al. 2008. Genetic diversity in Egyptian and Italian goat breeds measured with microsatellite polymorphism. Journal of Animal Breeding and Genetics **125**(3): 194-200.

Ajmone-Marsan, P., Negrini, R., Crepaldi, P., et al. 2001. Assessing genetic diversity in Italian goat populations using AFLP markers. Animal Genetics **32**(5): 281-288.

Angiolillo, A., Yahyaoui, M. H., Sanchez, A., et al. 2002. Short communication: Characterization of a new genetic variant in the caprine kappa-casein gene. Journal of Dairy Science **85**(10): 2679-2680.

Boulanger, A., Grosclaude, F., and Mahé, M. F. 1984. Polymorphism des caséines ás1 et ás2 de la chèvre (Capra hircus). Genetics Selection Evolution **16**: 157-176.

Bouniol, C., Brignon, G., Mahe, M. F., and Printz, C. 1994. Biochemical and genetic analysis of variant C of caprine alpha s2-casein (Capra hircus). Animal Genetics **25**(3): 173-177.

Caroli, A., Chiatti, F., Chessa, S., et al. 2006. Focusing on the goat casein complex. Journal of Dairy Science **89**(8): 3178-3187.

Clark, S., and Sherbon, J. W. 2000. Alpha(s1)-casein, milk composition and coagulation properties of goat milk. Small Ruminant Research **38**(2): 123-134.

Cosenza, G., Rando, A., Longobardi, E., et al. 1998. A MseI RFLP at the goat alpha s2-casein gene. Animal Genetics **29**(2): 150.

Flower, D. R. 1996. The lipocalin protein family: Structure and function. The Biochemical Journal **318**(Pt 1):1-14.

Folch, J. M., Coll, A., and Sanchez, A. 1994. Complete sequence of the caprine beta-lactoglobulin gene. Journal of Dairy Science **77**(12): 3493-3497.

Galal, S., Abdel-Rasoul, F., Anous, M. R., and Shaat, I. M. 2005. Onstation characterization of small ruminant breeds in Egypt. In Characterization of small ruminant breeds in West Asia and North Africa, edited by L. Iniguez. Syria: ICARDA, Aleppo. PP. 141-193.

Jann, O. C., Prinzenberg, E. M., Luikart, G., et al. 2004. High polymorphism in the kappa-casein (CSN3) gene from wild and domestic caprine species revealed by DNA sequencing. The Journal of Dairy Research **71**(2): 188-195.

Jànsa-Pérez, M., Leroux, C., Sanchez-Bonastre, A., and Martin, P. 1994. Occurrence of a LINE element in the 3'UTR of an allelic form of the goat alpha s1-casein gene associated with a reduced level of protein synthesis. Gene **147**: 179-187.

John, S. W., Weitzner, G., Rozen, R., and Scriver, C. R. 1991. A rapid procedure for extracting genomic DNA from leukocytes. Nucleic Acids Research **19**(2): 408.

Lagonigro, R., Pietrola, E., D'Andrea, M., et al. 2001. Molecular genetic characterization of the goat s2-casein E allele. Animal Genetics **32**(6): 391-393.

Mercier, J. C., Brignon, G., and Ribadeau-Dumas, B. 1973. Structure primaire de la caseine kappa B bovine. Sequence complete. (Primary structure of bovine kappa B casein. Complete sequence). European Journal of Biochemistry / FEBS **35**(2): 222-235.

Ng-Kwai-Hang, K. F. 1998. Genetic polymorphism of milk proteins: Relationships with production traits, milk composition and technological properties (Polymorphisme geìneìtique des proteìines du lait: Les relations entre les caracteìristiques de production, la composition du lait et les proprieìteìs technologiques). Canadian Journal of Animal Science **78**(Suppl): 131-147.

Pena, R. N., Saìnchez, A., and Folch, J. M. 2000. Characterization of genetic polymorphism in the goat â-lactoglobulin gene. Journal of Dairy Research **67**(2): 217-224.

Perez, M. D., and Calvo, M. 1995. Interaction of beta-lactoglobulin with retinol and fatty acids and its role as a possible biological function for this protein: A review. Journal of Dairy Science **78**(5): 978-988.

Prinzenberg, E. M., Gutscher, K., Chessa, S., et al. 2005. Caprine kappa-casein (CSN3) polymorphism: New developments in molecular knowledge. Journal of Dairy Science **88**(4): 1490-1498.

Ramunno, L., Cosenza, G., Pappalardo, M., et al. 2000. Identification of the goat CSN1S1F allele by means of PCR-RFLP method. Animal Genetics **31**(5): 342-343.

Ramunno, L., Cosenza, G., Pappalardo, M., et al. 2001. Characterization of two new alleles at the goat CSN1S2 locus. Animal Genetics **32**(5): 264-268.

Ramunno, L., Cosenza, G., Rando, A., et al. 2005. Comparative analysis of gene sequence of goat CSN1S1 F and N alleles and characterization of CSN1S1 transcript variants in mammary gland. Gene **345**(2): 289-299.

Ramunno, L., Longobardi, E., Cosenza, G., et al. 1999. A PstI PCR-RFLP at the goat CSN1S2 gene. Animal Genetics **30**(3): 242.

Rando, A., Ramunno, L., and Masina, P. 2000. Mutations in casein genes. Zoot.Nutr.Anim. **26**: 105-114.

Rijnkels, M. 2002. Multispecies comparison of the casein gene loci and evolution of casein gene family. Journal of Mammary Gland Biology and Neoplasia **7**(3): 327-345.

Roncada, P., Gaviraghi, A., Liberatori, S., et al. 2002. Identification of caseins in goat milk. Proteomics **2**(6): 723-726.

Schaar, J., Hansson, B., and Petterson, H. E. 1985. Effects of genetic variants of ê-casein and â-lactoglobulin on cheesemaking. Journal of Dairy Research **52**: 429-437.

Serradilla, J. M. 2002. The goat ás1-casein gene: A paradigm of the use of a major gene to improve milk quality? Séminaires Méditerraneéns **55**:99-106.

Threadgill, D. W., and Womack, J. E. 1990. Genomic analysis of the major bovine milk protein genes. Nucleic Acids Research **18**(23):6935-6942.

Vaiman, D., Schibler, L., Bourgeois, F., et al. 1996. A genetic linkage map of the male goat genome. Genetics **144**(1): 279-305.

Watson, C. J., Gordon, K. E., Robertson, M., and Clark, A. J. 1991. Interaction of DNA-binding proteins with a milk protein gene promoter in vitro: Identification of a mammary gland-specific factor. Nucleic Acids Research **19**(23): 6603-6610.

Yahyaoui, M. H., Angiolillo, A., Pilla, F., et al. 2003. Characterization and genotyping of the caprine kappa-casein variants. Journal of Dairy Science **86**(8): 2715-2720.

Yahyaoui, M. H., Coll, A., Sanchez, A., and Folch, J. M. 2001. Genetic polymorphism of the caprine kappa casein gene. The Journal of Dairy Research **68**(2): 209-216.

Yahyaoui, M. H., Pena, R. N., Sanchez, A., and Folch, J. M. 2000. Rapid communication: Polymorphism in the goat beta-lactoglobulin proximal promoter region. Journal of Animal Science **78**(4): 1100-1101.