# Genetic Polymorphism in β-lactoglobulin Gene of Some Goat Breeds in Egypt and its Influence on Milk Yield

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**Abstract:** Beta-lactoglobulin (*β*-LG) is one of the most important proteins in mammals' milk. It plays a crucial role in the milk quality. The polymorphism of *β*-LG gene can be used as a marker system. To determine the genotypes of *β*-LG gene and its influence on milk yield in some goat breeds reared at Sakha farm, Kafrelsheikh province, Egypt, 20 female goats representing four different breeds including; Egyptian Nubian (Zarayby), Damascus, Alpine and Balady Hybrid were used in this study. The obtained results showed that in Balady Hybrid and Zarayby breeds AB genotype were significantly ($p<0.05$) higher in milk production where in Damascus and Alpine AA genotype were significantly ($p<0.05$) higher in milk production. The sequence results revealed a substitution of G with A at nucleotide no. 6705 in both Balady Hybrid and Damascus breeds. Another substitution of G with A was recorded at nucleotide no. 6751 in the same breeds. Meanwhile, the sequence analysis of promoter region did not show any nucleotide differences among different breeds. Our results revealed that, the milk production may vary between different genotypes according to the breed.

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**Key Words**: Polymorphism, β-lactoglobulin, Goat, Milk Yield, Egypt.

## 1. Introduction

β-Lactoglobulin (*β*-LG) is an extremely acid stable protein with a molecular weight of 36,000 Daltons. The complete amino acid sequence of *β*-LG has been reported and genetic variation in amino acids sequence has been identified (Rachagani *et al.,* 2006). The bovine β-Lactoglobulin A variant differs from B variant by two amino acids only i.e. aspartate-64 and valine-118. These amino acids are substituted with glycine and alanine respectively in the B variant. All the variants contain five cysteine residues, four of which are involved in forming the intra-chain disulphide bridges. The biological functions of this protein are still not known. It could have a role in metabolism of phosphate in the mammary gland and the transport of retinol and fatty acids in the gut (Rachagani *et al.,* 2006). Polymorphism of β-LG had been investigated in cattle and sheep, in which it has a remarkable effect on milk yield and composition (Tsiaras *et al.,* 2005; Dario *et al.,* 2008). Similarly, in goat, several alleles had been discovered at both DNA and protein levels (Pena *et al.,* 2000; Yahyaoui *et al.,* 2000; Graziano *et al.,* 2003; Ballester, 2005). However, in goat, the effect of polymorphism of β-LG on milk yield is not clear.

In goats, no variants producing amino acid difference have been characterized at the DNA level. However, polymorphisms in the 3´untranslated region (exon 7) and in the *β*-LG proximal promoter region of Spanish and French goats have been described (Pena *et al.*, 2000; Yahyaoui *et al.*, 2000). Differences in *β*-LG content, ranging from 43% to 63% of major whey proteins have been detected in milk from Italian Girgentana goats (Chianese *et al.*, 2000). A polymorphism in the promoter region of individuals from this breed, with reduced *β*-LG content, has recently been identified but it was not correlated to the *β*-LG content (Graziano *et al.,* 2003).

B-LG gene polymorphism at the DNA level has been analyzed by PCR-RFLP (Pena *et al.,* 2000) and they reported two novel genetic variants in the 3' untranslated region (exon 7) of the β-LG gene. A single nucleotide polymorphism in the proximal region of the gene has been reported (Yahyaoui *et al.,* 2000). Ballester *et al.,* (2005) amplified and sequenced the proximal promoter and the first six exons containing the entire coding region for the β-lactoglobulin gene in eleven goat breeds from Spain, France, Italy, Switzerland, Senegal and Asia and detected fifteen polymorphisms, nine in the promoter region and six in the exons of the β-LG gene. Kumar *et al.,* (2006) reported genetic variants in Indian goat breeds as the already reported variants by Pena *et al.*, (2000) but no nucleotide sequence and thus no single nucleotide polymorphisms (SNPs) were reported in β-LG gene in Indian goat breeds.

The aim of this study was to detect polymorphism in the β-LG at both DNA levels in different breeds of goat. In addition, to analyze the effect of polymorphism on goat milk yield

## 2. Materials and Methods

***1- Blood samples and Genomic DNA extraction:***

Blood samples were collected from four goat breeds living in live stock management center belonging to the Sakha, Kafrelsheikh province, Egypt. Breeds included Zarayby, Damascus, Albino and Balady Hybrid. Blood samples were drawn from 5 animals from each breed into potassium EDTA evacuated blood collection tubes, transported to the lab in an ice box and then preserved in -20 °C till analysis. Genomic DNA was extracted from whole blood using EZ-10 spin column genomic DNA Minipreps kit (Bio Basic INC, Canada). DNA concentration and purity were examined using UV spectrophotometer at 260/280 nm.

***2- PCR Amplification of β-LG gene:***

Amplification of exon VII region from the *β*-LG gene was performed using polymerase chain Reaction (PCR). The PCR was carried out in a 50 µL reaction mixture containing: 100 ng genomic DNA, 0.5 *m*M of each primer, 1.0 U of *Taq* DNA Polymerase, dNTPs each at 150 mM and 5.0 µL of 10 × PCR buffer containing 1.5 mM $MgCl_2$. And 10 *p*Mol Forward primer: 5'- CGG GAG CCT TGG CCC TCT GG -3', Reverse primer: 5'- CCT TTG TCG AGT TTG GGT GT -3' (Pena *et al., 2000)*. The thermal cycling parameters were as follows: 35 cycles; denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min, followed by a final extension at 72°C for 7 min.

***3- Agarose gel electrophoresis:***

PCR products electrophoresis was performed using 15 µl of PCR products on 2% agarose (FMC, Rokland, ME) gel in 0.5X Tris-borate-EDTA buffer at 100 volt for 45 minutes. The gel was stained in 1% ethidium bromide and washed with distilled water. PCR products were visualized with a UV transilluminator and photographed using a UVP gel documentation system.

***4- Restriction enzyme digestion:***

PCR products were purified using BioFlux BioSpin Gel Extraction kit (Bioer technology Co., Ltd, Japan). A total volume of 10 µL of each purified PCR product was digested overnight at 37ºC with 10 U of *Sac*II endonuclease (Boehringer Mannheim, Germany). Amplicons as well as digested products were analyzed by electrophoresis in 3% agarose gel and then stained with 1% ethidium bromide.

***5- Exon VII and Promote region nucleotide sequence analysis:***

Nucleotide sequences were performed for both *β*-LG promoter and exon VII regions. For promoter region PCR was performed using primers 5́- GCA GGT GCT TGC AGA GCC -3́ from base no.1633 to number 1652 and reverse 5́- GCA ACC TAC CAC CCA CCC -3́ from base no.2316 to number 1633 with an expected size of 683 bp. For the exon VII region, primers 5́- AGG AAG TGG GTA CCT AAG GG -3́ from base no.6529 to number 6548 and reverse 5́- ATA CCG ACA GTA GTG GCT GG-3́ from base no.7211 to number 7191 with an expected size of 682 bp DNA samples were amplified for 33 cycles (94 °C one minute, 58 °C one minute, 72 °C one minute) with final elongation for five minutes at 72°C PCR products were purified using BioFlux BioSpin Gel Extraction kit (Bioer technology Co., Ltd, Japan). Sequencing reactions were performed in a MJ Research PTC-225 Peltier Thermal Cycler using an ABI PRISM BigDyeTM Terminator Cycle Sequencing Kits with AmpliTaq DNA polymerase (FS enzyme) (Applied Biosystems), following the protocols supplied by the manufacturer. Nucleotides sequences were analyzed and the phylogenic studies were conducted using DNasis program.

***6- Milk yield***

The records of milk production for the goats under the study were used to calculate the daily average milk production and the data was statistically analyzed using SPSS program using one way ANOVA. Data values represented in means ± SEM

## 3. Results

Amplification of exonVII of goat β-lactoglobulin gene produced single band with molecular size of 426 bp figure (1). Egyptian Nubian (Zarayby), Damascus, Alpine and Balady Hybrid breeds are shown on lanes 1, 2, 3 and 4 respectively. Digestion of PCR products with *S*acII endonuclease yielded 3 fragments. The first fragment was undigested product with molecular size of 426 bp which correspond to Alpine breed (Figure 2, lane 1); this undigested product was classified as $S_2S_2$ allele. The second fragment is a 349 bp, while the third fragment is a 77 bp. The second and third fragments were obtained with Damascus (lane 2), Egyptian Nubian (lane 3) and Balady Hybrid breed (lane 4); the second and third fragments were classified as $S_1S_1$ allele.

Milk yield of goats with genotype AB and AA is shown in table (1). In Egyptian Nubian breed, AB genotype had significantly higher milk yield ($p<$ 0.05) than AA genotype. However, In Alpine breed, the AA genotype had significantly higher milk yield ($p<$ 0.05) than the AB genotype. On the other hand, in Damascus and Balady Hybrid breeds, there was no significant difference between both genotypes within the same breed. Statistical analysis of data in the different groups of goats showed that, milk yield was significantly different ($p<0.05$) among different breeds. Nubian breed was the highest in milk yield; 1.79 ± 0.15 kg/day (a). Alpine breed was the next in daily milk yield recording 1.44 ± 0.12 kg/daily (b).

Damascus breed was the third in milk yield recording 0.87 ± 0.07 kg/day (c), while Balady Hybrid breed was the lowest in milk yield recording 0.22 ± 0.03 kg/day (d). Statistical analysis of data for comparing the mean daily milk yield between genotypes AB and AA showed that, there was no significant difference between both genotypes regardless of their breeds.

PCR amplification of exon VII region of *β*-LG gene, between nucleotide number 6529 and 7211, resulted in the same product size (682 bp) in all breeds under investigation (Fig.3a). The PCR products were subjected to sequence analysis in comparison to each other and to that published in gene bank (accession No. Z33881). The results revealed a substitution of G with A at nucleotide no. 6705 in both Balady and Damascus and breeds as well as intra-breed. Another substitution of G with A was found at nucleotide No 6751 in the same breeds. Meanwhile, the sequence analysis of promoter region did not showed any nucleotide differences among different breeds.

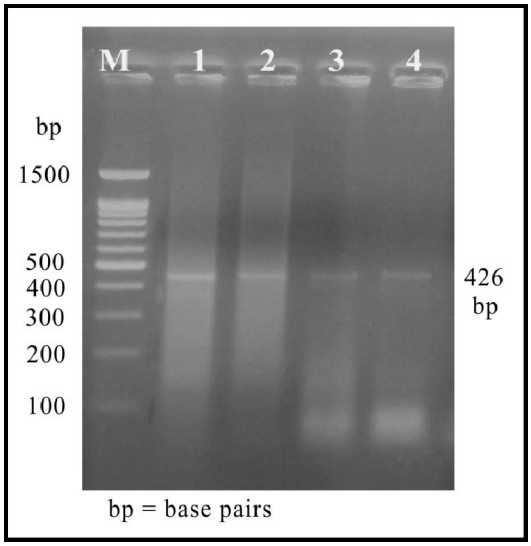


**Fig. 1:** PCR amplification of *β-LG* in goats under study. Lane 1is (Egyptian Nubian), lane 2 is (Damascus), lane 3 is (Alpine), lane 4 is (Balady Hybrid), M is (molecular weight marker). Amplified product is 426 bp.

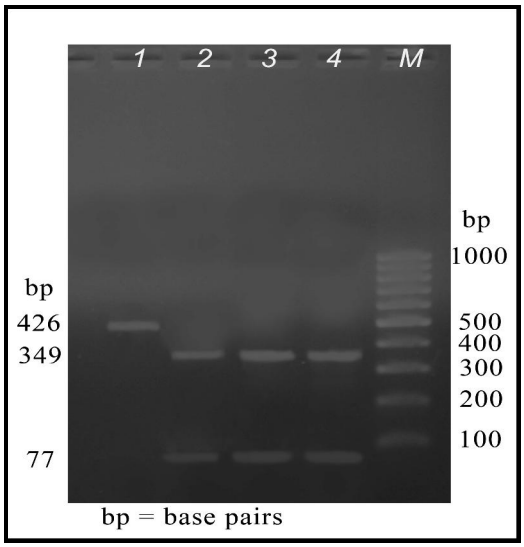


**Fig. 2**. Electrophoresis of exon VII of the caprine *β-LG* gene amplified by the polymerase chain reaction. Lane 1is (Alpine), lane 2 is (Damascus), lane 3 is (Egyptian Nubian), lane 4 is (Balady Hybrid), M is (molecular weight marker), digested with Sac II.

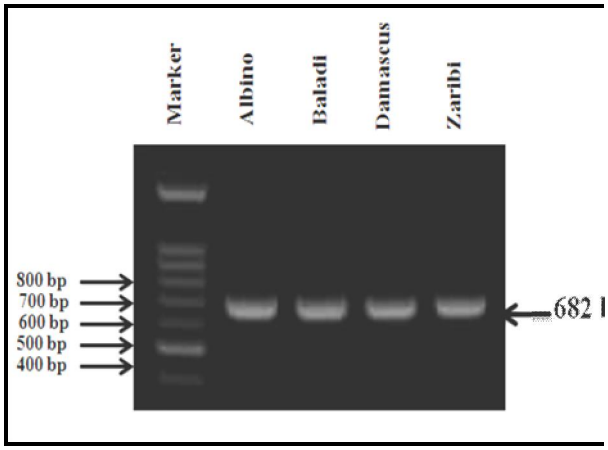


**Fig. 3.** Ethidium bromide stained agarose gel electrophoresis of PCR products from exon VII of ovine *β-LG* gene. M indicates 100 bp DNA size marker and other lanes are PCR products from different individuals from four breeds Albino, Balady, Damascus and Zaribi breeds.

**Table (1): Milk yield in relation to breed and genotypes of different goat breeds.**

| Breed | Genotype | | Overall breed means |
|---|---|---|---|
| | AB | AA | |
| **Nubian** | **2.01±0.43 *** | **1.58±0.16** | **1.79±0.15 a** |
| **Damascus** | **0.79±0.14** | **0.96±0.08** | **0.87±0.07 c** |
| **Alpine** | **1.27±0.19*** | **1.61±0.14** | **1.44±0.12 b** |
| **Hybrid Balady** | **0.32±0.09** | **0.13±0.03** | **0.22±0.03 d** |
| **Overall genotypes means** | **1.10±0.15** | **1.07±0.08** | |

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Gene bank   GGATCTGGCA GGTGCCCCAG GAATCACAGG GGGGGCCCAT GTCCATTTCA
Albino-1    GGATCTGGCA GGTGCCCCAG GAATCACAGG GGGGGCCCAT GTCCATTTCA
Albino-2    GGATCTGGCA GGTGCCCCAG GAATCACAGG GGGGGCCCAT GTCCATTTCA
Balady-1    GGATCTGGCA GGTGCCCCAG GAATCACAGG GGGGGCCCAT GTCCATTTCA
Balady-2    GGATCTGGCA GGTGCCCCAG GAATCACAGG GGGGGCCCAT GTCCATTTCA
Damascus-1  GGATCTGGCA GGTGCCCCAG GAATCACAGG GGGGGCCCAT GTCCATTTCA
Damascus-2  GGATCTGGCA GGTGCCCCAG GAATCACAGG GGGGGCCCAT GTCCATTTCA
Zaraibi     GGATCTGGCA GGTGCCCCAG GAATCACAGG GGGGGCCCAT GTCCATTTCA

                                                        *
Gene bank   GGGCCCGGGA GCCTTGGCCC CTCTGGGGAC AGACGACGTC ACCCCCGCCT
Albino-1    GGGCCCGGGA GCCTTGGCCC CTCTGGGGAC AGACGACGTC ACCCCCGCCT
Albino-2    GGGCCCGGGA GCCTTGGCCC CTCTGGGGAC AGACGACGTC ACCCCCGCCT
Balady-1    GGGCCCGGGA GCCTTGGCCC CTCTGGGGAC AGACGACATC ACCCCCGCCT
Balady-2    GGGCCCGGGA GCCTTGGCCC CTCTGGGGAC AGACGACGTC ACCCCCGCCT
Damascus-1  GGGCCCGGGA GCCTTGGCCC CTCTGGGGAC AGACGACATC ACCCCCGCCT
Damascus-2  GGGCCCGGGA GCCTTGGCCC CTCTGGGGAC AGACGACGTC ACCCCCGCCT
Zaraibi     GGGCCCGGGA GCCTTGGCCC CTCTGGGGAC AGACGACGTC ACCCCCGCCT

                                                    **
Gene bank   CCCCCATCAG GGGGACCAGG AGGGACCGGG ACCGCGGTCA CCTCTCCTGG
Albino-1    CCCCCATCAG GGGGACCAGG AGGGACCGGG ACCGCGGTCA CCTCTCCTGG
Albino-2    CCCCCATCAG GGGGACCAGG AGGGACCGGG ACCACGGTCA CCTCTCCTGG
Balady-1    CCCCCATCAG GGGGACCAGG AGGGACCGGG ACCGCGGTCA CCTCTCCTGG
Balady-2    CCCCCATCAG GGGGACCAGG AGGGACCGGG ACCACGGTCA CCTCTCCTGG
Damascus-1  CCCCCATCAG GGGGACCAGG AGGGACCGGG ACCACGGTCA CCTCTCCTGG
Damascus-2  CCCCCATCAG GGGGACCAGG AGGGACCGGG ACCGCGGTCA CCTCTCCTGG
Zaraibi     CCCCCATCAG GGGGACCAGG AGGGACCGGG ACCGCGGTCA CCTCTCCTGG

Gene bank   GACCCAGGCC CCTCCAGGCC CCTCCTGTGG CCTCCTGCTC GGGGCCGCTC
Albino-1    GACCCAGGCC CCTCCAGGCC CCTCCTGTGG CCTCCTGCTC GGGGCCGCTC
Albino-2    CACCCACCCC CCTCCACCCC CCTCCTCTCC CCTCCTCCTC CCCCCCCCTC
Balady-1    GACCCAGGCC CCTCCAGGCC CCTCCTGTGG CCTCCTGCTC GGGGCCGCTC
Balady-2    GACCCAGGCC CCTCCAGGCC CCTCCTGTGG CCTCCTGCTC GGGGCCGCTC
Damascus-1  GACCCAGGCC CCTCCAGGCC CCTCCTGTGG CCTCCTGCTC GGGGCCGCTC
Damascus-2  GACCCAGGCC CCTCCAGGCC CCTCCTGTGG CCTCCTGCTC GGGGCCGCTC
Zaraibi     GACCCAGGCC CCTCCAGGCC CCTCCTGTGG CCTCCTGCTC GGGGCCGCTC
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Fig. 4. Panel A: Sequence analysis of the four different breeds comparing with that cited in gene bank. (*)Substitution at nucleotide number 6705 while (**) indicated the substitution at nucleotide 6751

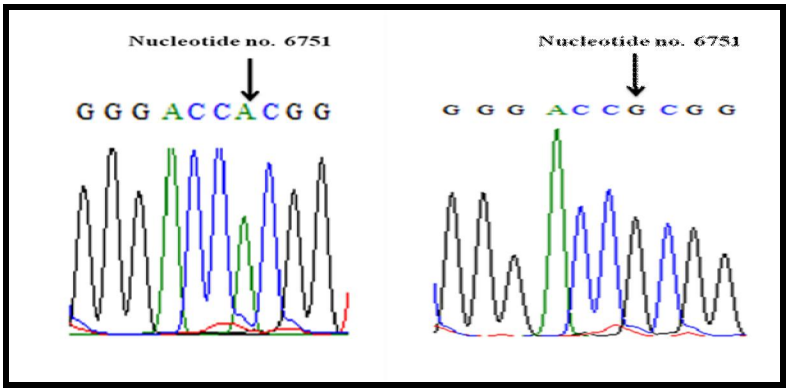


Fig. 4. Panel B. Sequence electrophenogram is showing the nucleotide substitution.

## 4. Discussion

Goats have played an important role in food culture since ancient time. They are also one of the oldest domesticated animals. Goat's milk has the advantage of being easier to digest; this is in part because the protein curds formed in the stomach are softer than that of cow's milk. This, in turn, makes digestion faster and easier than cow's milk. The occurrence of genetic polymorphism in milk proteins was described for the first time by Aschaffenburg and Drewry (1955). Since then, many studies have been performed to investigate milk protein polymorphism and great progress has been made with the introduction of analytical techniques that offer a greater degree of resolution, especially since the advent of DNA and protein analysis methods. This has led to an extensive investigation of genetic polymorphism of the six main milk proteins, including β-lactoglobulin, and to the identification of several variants (Ng-Kwai-Hang *et al.,* 1998; Martin *et al.,* 2002). Currently, genetic polymorphisms are playing an increasingly important role as genetic markers in many fields of animal breeding. With the development of molecular genetic techniques, it has become possible to establish a new class of gene markers based upon the variability at DNA sequence level (Meignanalakshmi and Nainar, 2009). In the recent years a significant progress has occurred in understanding many of the complex processes in the body cells at a molecular level. To clarify the basis of this phenomenon the development in the field of Molecular Biology was a great contribution. Goat's *β*-LG gene is located on chromosome 11q28. It was reported that, there is a high homogeneity of β-lactoglobulin in bovine, ovine and goat. Sheep and goat β-lactoglobulin differs from bovine *β*-LG only in 6 positions (Strzelec & Niżnikowski, 2009; Yahyaoui *et al.,* 2000). The reported relationship between genetic variants of the *β*-LG and milk yield, milk composition and cheese-making ability in cattle have raised interest for the establishment of the relationship between *β*-LG polymorphism and milk production traits in dairy goat and sheep populations (Vlatka Cubric-Curiket *et al.,* 2002). The effect of milk protein polymorphism on milk production traits has been investigated during the past decades and in some cases results were conflicting (Prinzenberg *et al.*, 2003; Kucerova *et al.,* 2006). In sheep, some studies observed that *β*-LG polymorphism significantly affects milk yield (Bolla *et al.,* 1989; Faraghì *et al.,* 1996), fat and protein content (Garzon and Martínez, 1992), cheese yield and composition (Di Stasio *et al.,* 1997; Rampilli *et al.,* 1997) and only fat content (Pirisi *et al.,* 1999). However, other studies failed to detect any effect of genetic polymorphism on milk production traits (Barillet *et al.,* 1993; Recio *et al.,* 1997; Piwczynski *et al*., 2002).

In the present study, the relationship between the *β*-LG genotype and milk production has been investigated. The data showed conflicting results as in other species. The study cannot find a clear relationship between genotypes and the milk yield as each breed has its specific relationship. In Nubian and Hybrid breed the AB genotype was superior to AA genotype in milk yield. In the contrary, both Damascus and Alpine breeds the AA genotype has this superiority.

Concerning Damascus an Alpine breeds similar results were observed in all the individual milk samples from the Indian goat where Kumar *et al.,* ( 2006) showed that, β-LG AA genotype had a higher milk yield than the β-LG AB genotype in Barbari and Jamunapari goats. A similar observation has been made by Prakash *et al.,* (2002) in 5 Indian goat breeds. The association of *β*-LG polymorphism with milk yield has been reported in cows and sheep (Ng-Kwai-Hang 1998; Moili *et al.,* 1998). Also, El-Hanafy *et al.,* (2010), found that, the frequency of AA genotype was higher in Damascus breed than Barki and Damascus x Barki crossbred and according to reference, milk production was associated with significantly higher milk production in this goat breed as compared by the two other goat breeds.

Although the β-lactoglobulin polymorphism and its effect on milk production and cheese characteristics have been extensively studied, the results are often contradictory indicating the predominance of the *β*-LG genotype or the absence of any effect (Amigo *et al.,* 2000)

Concerning Nubian and Hybrid breed, the findings are supported by the results of Barillet *et al.,* (1993), they showed a predominance of AB genotype with the highest daily milk production, followed by AA being also superior to BB genotype.

The results of this study indicate that, the relationship between β-LG polymorphism and milk yield is conflicting and may depend on the breed under the study. This is may be useful in using the high milk yield genotype in breeding inside each breed. Also more investigation including large number of animals from each breed must be investigated.

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