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POLYMORPHISM OF β-LACTO GLOBULIN GENE IN BARKI AND DAMASCUS AND THEIR CROSS BRED GOATS IN RELATION TO MILK YIELD

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Abstract: β-LG gene polymorphism at the DNA level has been analyzed by PCR-RFLP and reported two novel genetic variants of the β-LG gene in Indian goats. However, no study has been reported in Barki Egyptian local goat breed, Damascus and their crossbred goats regarding β-LG polymorphism at DNA level. The association of β-LG polymorphism with milk yield has been reported in cows, sheep and Indian goats. However, this relation has not clarified yet in local or foreign goat breeds raised in Egypt. This study aimed to fingerprint β-LG gene in the Barki, Damascus and their crossbred goats in an attempt to have clear image about genotypes of β-LG gene in these breeds in relation to their milk yield. A total of 120 adult female does belonging to these three different genetic groups were analyzed by the PCR-RFLP method. The goats are raised by farmers in North West coast (Borg EL-Arab, Alexandria, Egypt) in which goats are living in natural habitats. The amplified product was observed as 426 bp and the restriction digestion with *Sac*II revealed three genotypes, namely AA, AB and BB at the β-LG locus. The frequencies of AA, AB and BB genotypes were 0.1, 0.8, 0.1; 0.85, 0.1, 0.05; 0.41, 0.51, 0.08 in the Barki and Damascus and their crossbred goats, respectively. 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.

Key words: β-LG gene, PCR-RFLP, goat, polymorphism, milk yield

Introduction

Goats are the most versatile domestic animals in adaptation to arid and humid, tropical and cold, and desert and mountain conditions *(Gall, 1991; Quartermain, 1991; Silanikove, 2000)*. Goat milk is characterized with its offensive odor. This is especially from buck whose odor floats strongly around the premises and can affect the flavour of the milk. The unpleasant odor is obvious in milk if ventilation, milking practices and cooling of milk are improper or insufficient. Recently milked and cooled goat milk is odor free and hard to distinguish from cow milk in odor and taste *(Mowelm, 1988)*. *Gall (1996)* provides detailed description and production data of 160 goat breeds based on size of population, productivity and unique characteristic. Milk yield production data vary much from country to country for the same breed, depending on feeding, climate and adaptation to diseases.

The importance of goats as a potential source of meat and milk has been realized particularly in small farming system in developing countries. The small breeder (Bedouin) of the western desert of Egypt depends on goats solely for milk and with sheep for meat production. In Egypt, there are three indigenous goat breeds: Baladi (local breed in Delta), Barki (local breed in Desert) and Zaraibi. They are dual- purpose animals, with does kept for milk and bucks for meat *(Latif et al., 1987)*.

In Egypt, goats are an important source of meat. They are distributed across the country, especially dense in the Nile valley and delta and with lower concentration in the north-western coastal region and at oases *(Galal et al., 2005)*. In the Nile valley goats are usually found in small holdings as mixed flocks with sheep and other farm animals like cattle and buffaloes, while in the north-western Mediterranean coast they are in large herds mixed with sheep.

Barki desert goat is the only goat breed raised in the coastal zone of the western desert in Egypt where it represents 10% of the respective total goat population in Egypt. It is known to be hardy and well adapted to harsh environmental conditions and the scarce vegetation of the desert *(Haider, 1982)*. However, it is the most of the subtropical breeds of goats have poor kid performance, low milk yield with high fertility and moderate prolificacy *(Eissa, 1996)*. Improving goat productivity in subtropical countries was frequently suggested to be achieved either through selection within native breeds or through crossing with improved exotic breed *(Aboul – Naga et al., 1985; Abdelaziz et al., 1995)*. Recently, more attention is given to improve milk quantity and quality by crossbreeding local breeds with Damascus breed which considers as an improved breed *(Abdelsalam et al., 2000)*.

Most of the livestock breeds in Egypt lack molecular characterization required for establishing adequate utilization of genetic variation in developing animal production. Goat genetic improvement schemes in Egypt have involved crossbreeding trials with examples Damascus goats and the development of local breeds, in which the Zaraibi breed has been a recent target of joint work with the Food and Agriculture Organization of the United Nations (FAO). Goats are spread over a wide range of habitats with a substantial concentration in the tropics and dry zones in developing countries *(Galal, 2005; FAOSTAT 2006)*. Therefore, they are expected to show a large amount of genetic diversity in adapting to the varying ecosystems.

Farm animal genetic diversity is required to meet current production needs in various environments, to allow sustained genetic improvement, and to facilitate rapid adaptation to changing breeding objective *(Crawford and Littlejohn, 1998; Kumar et al., 2006b; El-Hanafy and Salem, 2009; El-Hanafy and El-Saadani, 2009)*.

Extensive polymorphism is present in structural genes mostly with co-dominant expression. Beta-lactoglobulin (β-LG) is a major whey protein in the milk of ruminants. It is also found in the milk of other mammals, but absent from the milk of rodents, lagomorphs or humans *(Hambling et al., 1992)*. A large number of different variants have been identified both within and between populations in the α s_1, α -s_2, β- casein and κ- casein loci *(Grosclaude et al., 1987)*. The existence of genetic polymorphism at the β- LG locus came into light in the first report on the occurrence of two forms of beta-lactoglobulin (β- LG) in cows by Aschaffenburg and *Drewry (1955)*. The electrophoretic pattern of the β-LG gene in goats has been analysed by SDS-PAGE by different workers *(Grosclaude et al., 1987; Prakash et al., 2002)*. β-LG gene polymorphism at the DNA level has been analyzed by PCR-RFLP and reported two novel genetic variants of the β -LG gene in Indian goats *(Kumar et al., 2006a)*. However, no study has been reported in Barki Egyptian local goat breed, Damascus and their crossbred goats regarding β-LG polymorphism at DNA level. The association of β-LG polymorphism with milk yield has been reported in cows, sheep and Indian goats. However, this relation has not clarified yet in local or foreign goat breeds raised in Egypt. This study aimed to fingerprint β-LG gene in the Barki, Damascus and their crossbred goats in an attempt to have clear image about genotypes of β-LG gene in these breeds in relation to their milk yield and this would be beneficial for improving milk productivity of local Egyptian breeds such as Barki throughout breeding program in which identified superior genotypes of local and exotic breeds may be spread by optimal crossbreeding with local animal with minimum effects on the adaptation traits of the local animal breeds.

Materials and Methods

Animal materials and DNA extraction. The present study was conducted on a total of 120 animals belonging to Barki, Damascus and their crossbred goats, viz. Barki (40), Damascus (40), 1/2 Damascus x 1/2 Barki (40). Blood samples were taken randomly from forty adult female does for each of the three breeds. The goats are raised by farmers in North -west coast (Borg EL-Arab, Alexandria, Egypt) in which goats are living in natural habitats. Approximately, 10 ml venous blood was collected from each animal using 0.5 ml of 2.7% EDTA as an anticoagulant. Genomic DNA was isolated from blood using DNA extraction kit (GF-1, Vivantis) according to the manufacturer's instructions. The quality of DNA was checked by spectrophotometry taking ratio of optical density (OD) value at 260 and 280 nm. Good quality DNA having OD ratio between 1.7 and 1.9 was used for further work. The poor quality DNA was re-extracted with phenol – chloroform.

PCR- RFLP of β-LG gene. Amplification of the β-LG gene from exon 7 to the 3' flanking region was amplified from genomic DNA. The PCR was carried out in a 25 µL reaction mixture containing: 100-150 ng genomic DNA, 0.5 µM of each primer (forward: F F5'-CGG GAG CCT TGG CCC TCT GG -3', reverse: 5'-CCT TTG TCG AGT TTG GGT GT-3'), 1.00 U of Taq DNA Polymerase, 2.5 µL of 10 × PCR assay buffer (1.5 mM MgCl2), dNTPs each at 100 µM *(Pena et al., 2000)*. The amplification was carried out using a pre-programmed thermal cycler (Eppendorf Mastercycler) with the following conditions: an initial cycle 95°C for 5 min, 35 cycles of steps containing denaturation, 95°C for 30 s, annealing at 60°C for 1 min and extension at 72°C for 60 s and final extension at 72°C at 5 min.

Restriction enzyme digestion: A total volume of 15 β-LG of each PCR product was digested with 10 U of *Sac*II endonuclease for 9 hours at 37ºC. PCR and digested products were analyzed by means of electrophoresis in 3% agarose gel stained with ethidium bromide. The digested products were visualized and documented under gel documentation system (Syngene).

Genotype analysis. The PCR of the β-LG gene produced a 426 base pair (bp) band. After digestion with *Sac*II endonuclease, the β- LG gene homozygous carriers had two bands at a 349 and 77 bp (BB), the noncarrier had undigested 426 bp band (AA), whereas heterozygotes had both 426 , 349 and 77 bp bands (AB).

Results and Discussion

Figure 1 represents agarose gel electrophoresis of PCR amplified β-LG gene (426 bp) of Barki and Damascus and their crossbred goats, while Figure 2 represents different genotypes (AA, AB, BB) resulted from *Sac*II digested β-LG gene product of these breeds.

Genotyping at the β-LG locus at the DNA level revealed the presence of two alleles (A, B). The region spanning from exon 7 to the 3'flanking region of the goat β-LG gene was amplified from a genomic DNA sample belonging to the three genetic groups. The PCR amplified product was observed as 426 bp (Figure 1). The PCR product was digested with *Sac*II and subsequently analyzed for mutation in the fragment. Mainly three genotypes, namely noncarrier had undigested 426 bp band (AA), homozygous carriers had 349 and 77 bp bands (BB) and heterozygotes had both 426, 349 and 77 bp bands (AB) were observed in the population (Figure 2).

PCR + RFLP with the *Sac*II enzyme recognized the polymorphic site, which was produced by a single nucleotide substitution in position +4601 *(Pena et al., 2000)*. The allelic and genotypic frequencies of the β-LG gene polymorphism for the Barki , Damascus goats and their crossbred with reference milk production are presented in Table 1. The frequencies of AA, AB, BB genotypes are 0.1, 0.8, 0.1; 0.85, 0.1, 0.05; 0.41, 0.51, 0.08 for Barki, Damascus goats and their crossbreds, respectively. The allelic frequencies (A, B) are 0.50, 0.50; 0.9, 0.1; 0.66, 0.33 for Barki, Damascus goats and their crossbreds, respectively.

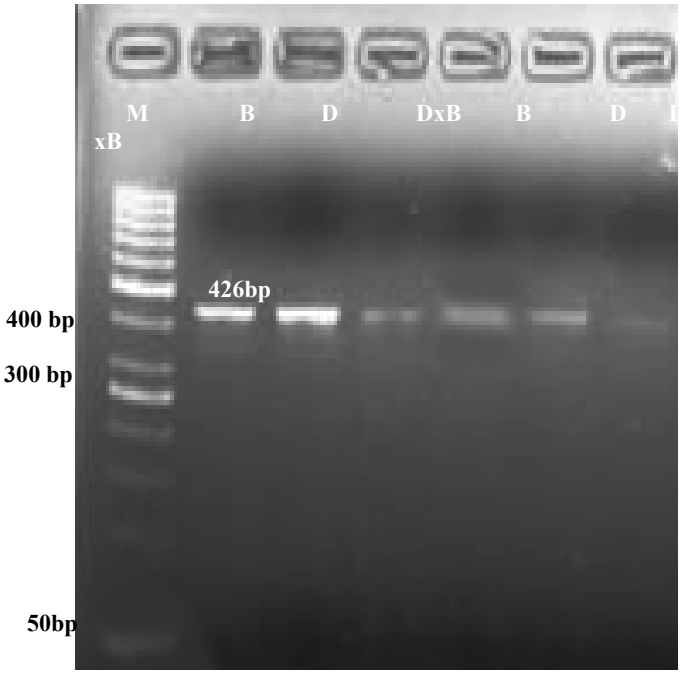

Figure 1. PCR amplification of β-LG gene (426 bp,Lanes 2-7) in Barki (B), Damascus (D) and Damascus x Barki (DxB) goat breeds . Lane M, molecular size marker (50 bp DNA ladder)

It is obvious from these findings that Damascus breed has the highest AA frequency (0.85), while the Barki breed has the lowest one (0.1) and Damascus x Barki, crossbred has intermediate value for this genotype (0.41). Regarding AB frequency Barki breed has the highest frequency (0.80), while Damascus breed has the lowest one (0.1) and Damascus x Barki, crossbred has intermediate value for this genotype (0.51). In respect to BB genotype, it has the lowest genotype frequency and ranged from 0.05 to 0.1 in the two breeds and their crossbred. The allelic frequencies has the same trend in the two breeds and their crossbred, e.g. Damascus breed has the highest (A) frequency (0.9) while the Barki breed has the lowest one (0.5) and Damascus x Barki crossbred has intermediate value for this allele (0.66).

Table 1. Genotypic and allelic frequencies after *Sac*II digestion of fragment obtained from PCR of the DNA region spanning from Barki, Damascus and Damascus x Barki goat breeds and their related reference milk production

Breed	N	Genotypic frequency			Allelic frequency		Milk yield (kg/lactation season)*
		AA	AB	BB	A	B	
Barki	40	0.1	0.8	0.1	0.5	0.5	91.1
Damascus	40	0.85	0.1	0.05	0.9	0.1	174.1
Damascus x Barki	40	0.41	0.51	0.08	0.66	0.33	113.1

**** Abdelsalam et al. (2004)***

According to literature Damascus breed had significantly highest milk production (174.1 kg/ lactation season) under desert farm condition, while Barki breed has the lowest milk production (91.1 kg/ lactation season) and Damascus x Barki crossbred has intermediate milk production (113.1 kg/ lactation season) at the same condition *(Abdelsalam et al., 2004)*.

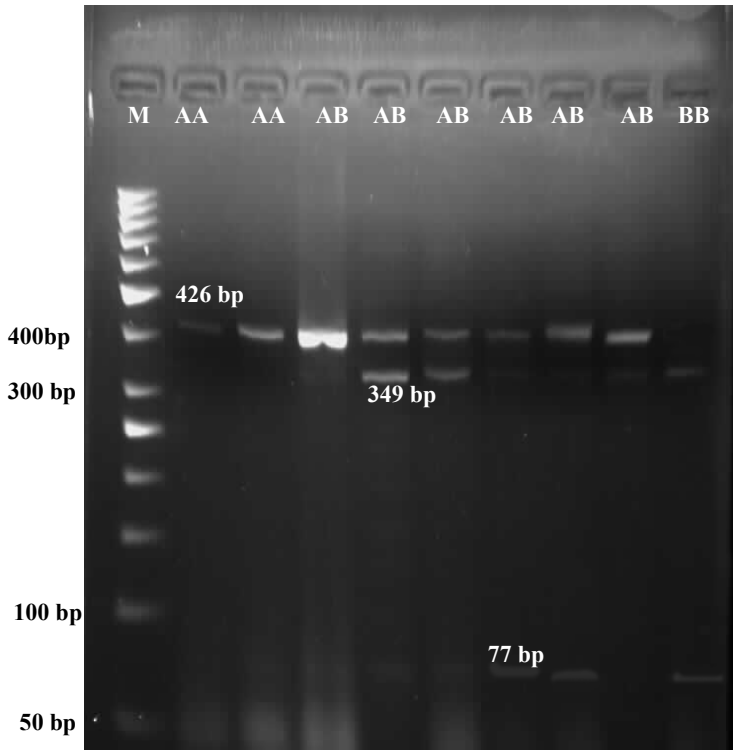

Figure 2. Electrophoresis of PCR-RFLP of caprine β-LG gene after digestion by *Sac*II of animals with AA, AB, BB genotypes. Lane M, molecular size marker (50 bp DNA ladder)

By correlating reference milk production of these breeds with allelic frequencies of the β-LG gene polymorphism obtained from this study, it is easily to note that Damascus breed had the highest milk production and also had highest AA frequency (0.85), while Barki breed had the lowest milk production and also had the lowest one (0.1) and Damascus x Barki crossbred had intermediate milk production , AA frequency (0.41). On the other hand, the highest heterozygotes (AB) frequency (0.8) in Barki breed was accompanied by the lowest milk production among Barki, Damascus goats and their crossbreds and vice versa for Damascus goats breed, while Damascus x Barki, crossbred lay on intermediate value.

These findings is in accordance with the findings of *Kumar et al. (2006a)*. They found that The β-LG genotype had a significant effect on milk yield at 90 days in both Jamunapari and Barbari goats. B-LG AA had a higher milk yield than β-LG AB in both Jamunapari and Barbari breed ($P<0.01$). The milk yield at 90

days was 81.82 ± 2.21 and 68.97 ± 3.86 liters in the β-LG-AA and β-LG-AB genotype, respectively, in Barbari goats. Similarly, in Jamunapari goats the milk yield was 97.69 ± 2.83 and 85.35 ± 2.37 liters in the β-LG-AA and β-LG-AB genotype, respectively. On the other hand, the genotype frequencies obtained in the present study is differ from that of *Kumar et al. (2006a)*. They analyzed genotype frequencies in 13 genetic groups of Indian goat and found that BB proportion was too high and varied from 41.7% to 100%. Similarly, the AB proportion varied from 3.6% to 94% in different breeds. Surti and Marwari had a lower AB genotypic frequency in the population. The AB genotypic frequency was highest (0.994) in Gaddi samples.

The superiority of AA genotype in goat milk production observed in the present study was also noted by *Prakash et al. (2002)* in 5 Indian goat breeds. However in other study *Moili et al. (1998)* found that milk from heterozygous (AB) ewes had higher casein contents with higher yields than homozygous AA or BB ewes. In cows, the B allele was associated with a lower whey protein yield (Ng-Kwai-Hang, 1998). Polymorphic sites have been identified in Barki, Damascus and their crossbred goats in exon 7 of the caprine β-LG gene as observed in other goats of France and Spain *(Folch et al.,* 1999*; Pena et al. 2000)*. *Boulanger (1976)* observed A and B variants in five Alpine and Sannen goat breeds at the β -LG locus. Similar results were observed in all the individual milk samples of the Indian goat. Similarly, three genotypes were observed in Indian goats at the β-LG locus. On contrary to the present study was the most frequent genotype in the entire studied sample. It was stated that caprine - S1 casein and bovine β-LG and κ-casein have affected the physicochemical properties of milk, protein contents and cheese making properties *(Ng-Kwai-Hang, 1998)*.

Regarding polymorphism off β-LG in other species eight variants have been reported at the DNA level at the bovine β-LG locus, however, alleles A and B are most frequent *(Ng-Kwai-Hang, 1998)* and allele B was associated with a lower whey protein content and a higher casein content in milk *(Ng-Kwai- Hang, 1998)*. In sheep, three variants (A, B & C) have been reported *(Erhardt, 1989)*. The β-LG locus in the goat had been characterized at the DNA level and two new genetic variants at the β-LG LG locus had been reported *(Pena et al., 2000)*.

In the present study PCR amplification and RFLP analysis were found to be a rapid and sensitive method to identify β-LG genotypes, directly at the DNA level without milk samples of lactating females. In addition, β-LG genotyping can be used in selecting superior genetic structures for milk production in young female goats in shorter time than the traditional election could. The selection of these superior individuals in early age and culling of the lower ones based on their genetic structures could participate in improving milk production from local adaptive goats such as Barki breed and can help in determine the optimum ratio of crossbreeding with superior genotypes of local and foreign breeds that can improve milk production trait in local animals with minor effect on their adaptive characteristics.

Conclusion

A total of 120 individuals belonging to these three different genetic groups mainly Barki, Damascus and their crossbred were analyzed for β-LG polymorphism at DNA level by the PCR-RFLP method. The amplified product was observed as 426 bp and the restriction digestion with *Sac*II revealed three genotypes, namely AA, AB and BB at the β-LG locus. The frequencies of AA, AB and BB genotypes were 0.1, 0.8, 0.1; 0.85, 0.1, 0.05; 0.41, 0.51, 0.08 in the Barki and Damascus and their crossbred goats, respectively. 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. In addition, β-LG genotyping can be used in selecting superior genetic structures for milk production in young female goats in shorter time than the traditional selection could. The selection of these superior individuals in early age and culling of the lower ones based on their genetic structures could participate in improving milk production from local adaptive goat breeds.

Further studies should be made using larger number of animals from more goat breeds and different geographic region in Egypt in order to get more precious genotyping and to determine required crossbreeding ratio with superior individuals of local and foreign goat breeds carry the favorite genotypes of this gene which will lead to increase of expression of these gene in the local Egyptian goat breeds without affect on the acclimatization traits of these breeds to the environmental conditions in Egypt.

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Polimorfizam β-lakto globulin gena kod koza rase barki i damask i njihovih meleza u odnosu na prinos mleka

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Rezime

Veza između β-LG polimorfizma i prinosa mleka je utvrđena kod krava, ovaca i indijskih koza. Međutim, ovaj odnos još uvek nije objašnjen kod koza

lokalnih ili stranih rasa koje se gaje u Egiptu. Ovo istraživanje je imalo za cilj dešifrovanje β-LG gena koza rase barki, damask i njihovih meleza u pokušaju da se dobije jasna slika o genotipovima β-LG gena kod ovih rasa u odnosu na prinos mleka, što bi bilo korisno za poboljšanje produktivnosti lokalnih egipatskih rasa, kao što je barki, u okviru odgajivačkog programa gde bi se otkrili superiorni genotipovi egzotičnih rasa mogu ubrzani kroz optimalno ukrštanje sa lokalnim grlima sa minimalnim uticajima na osobine adaptacije lokalnih rasa. Ovo istraživanje je izvedeno na 120 koza rase barki i damask, kao i njihovih meleza, barki (40), damask (40), 1/2 damask x 1/2 barki (40). β-LG gen od eksona 7 do 3' slobodnog bočnog regiona je pojačan iz genomske DNK korišćenjem specifičnih prajmera i nakon digestije sa *Sac*II endonukleazom, homozigotni nosioci β-LG gena su imali dve trake na 349 i 77 bp (BB), ne-nosioci su imali nepresečenu endonukleazu 426 bp traku (AA), dok su heterozigoti imali i 426, 349 i 77 bp trake (AB). Frekvencije AA, AB i BB genotipova su bile 0,1, 0,8, 0,1; 0,85, 0,1, 0,05; 0,41, 0,51, 0,08 kod rasa barki i damask i njihovih meleza, respektivno. Frekvencija (AA) genotipa je bila viša kod koza rase damask nego kod barki koza, i meleza damask x barki, a u vezi sa proizvodnjom mleka, ona je bila značajno viša kod koza ove rase u poređenju sa kozama druge dve rase.

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