

## POLYMORPHISM OF B-LACTO GLOBULIN GENE IN BARKI SHEEP BREED

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**Abstract:**  $\beta$  - LG gene polymorphism at the DNA level has been analyzed by PCR-RFLP and reported two genetic variants of the  $\beta$  -LG gene in Pag native and Leccese sheep breeds. However, no study has been reported in Barki Egyptian local sheep breed regarding  $\beta$  -LG polymorphism at DNA level. The association of  $\beta$ - LG polymorphism with milk production and manufacturing properties of the cheese in sheep have been extensively studied. However, the results are conflicting, indicating either superiority of a given  $\beta$  - LG variant or absence of relationship. The fingerprinting of  $\beta$  -LG gene and this relation has not clarified yet in local Barki and other sheep breeds raised in Egypt. Regarding the importance of Barki sheep breed as a local adaptive breed in Egypt, this preliminary study aimed to fingerprint  $\beta$ -LG gene in the Barki sheep breed, in an attempt to have clear image about genotypes of  $\beta$ -LG gene in this breed. A total of 60 adult females Barki ewes were fingerprinted for  $\beta$ -LG gene by the PCR-RFLP method. The sheep are raised by farmers in North West coast (Borg EL-Arab, Alexandria, Egypt) in which sheep are living in natural habitats. The amplified product was observed as 120 bp and the restriction digestion with *Rsa* I revealed three genotypes, namely AA, AB and BB at the  $\beta$ -LG locus. The frequencies of AA, AB and BB genotypes were 0.45, 0.30, 0.25, respectively.

**Key words:**  $\beta$  - LG gene; PCR-RFLP; Barki sheep; Polymorphism, milk yield

## Introduction

Sheep contribute 6% of the total red meat produced in Egypt. Regarding the economic importance of sheep in meat production in Egypt, it becomes essential to make fingerprinting of some genes related to economic traits such as litter size and growth rate, milk yield in order to determine the polymorphism pattern of these genes in the Egyptian sheep breeds. Determination of the genetic diversity of indigenous sheep in Egypt in respect to these important economic genes has not been sufficiently studied. Genetic characterization and determination of genetic differences between and within sheep breeds will help in the genetic improvement programs. In Egypt, most commercial sheep are raised in very small flocks in low-input systems. Genetic improvement is largely accomplished through government-owned flocks, with progeny from these flocks distributed to producers. Breeding objectives are needed to develop selection programs for these breeds (*Almahdy et al.*, 2000)

In Egypt, there are almost 5.2 million head of sheep (FAO, 2007) out of which one third is maintained in the western desert especially in the north western coastal area. Barki sheep, which is the dominant coarse wool fat-tailed breed of this area, is known to be well adapted to the harsh prevailing conditions including poor feeding, heat stress and disease. The breed is considered the main contributor to the livestock population in the Mediterranean area. They are multi-colored, usually white with brown or black head and legs. They produce coarse wool and have a small fat tail. They are the smallest Egyptian breed. Barki sheep are distributed along a belt on the Mediterranean sea coast of the western desert for a length of about 500 km with a depth of about 25 km. Sheep is considered the main contributor to livestock population in the area, where the annual rainfall ranges from 150 to 180mm. Animals graze natural vegetation during a limited grazing season which extends from November to March. Rain fed barley is cultivated and consumed by both inhabitants and their animals especially during the long dry season which extends from April to November. Barki sheep produce lamb and wool. The milk production of ewes is low and is hardly sufficient for lamb suckling. It was reported (*EI-Shahat*, 1970) that Barki ewes produced an amount of 40.6 kg of milk, in a lactation period of 18 weeks, using the hand milking technique, with averages of 5.1 and 5.4 of fat and protein percent, respectively. In a survey of some Barki flocks, in the north western coastal zone, *Aboul-Naga et al.* (1985) reported that only 26% of flock owners milk their ewes, at 3 months post lambing, for six weeks. Ewes when milked are mostly once daily to give an average daily milk yield of 0.3 kg.

Most of the livestock breeds in Egypt lack molecular characterization required for establishing adequate utilization of genetic variation in developing animal production. 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.*, 2006; *EL Hanafy and Salem*, 2009; *EL Hanafy and EL- Saadani*, 2009; *EL Hanafy et al.*, 2010).

Although in recent years the effects of  $\beta$ -LG polymorphism on sheep milk production and manufacturing properties of the cheese have been extensively studied, the results are conflicting, indicating either superiority of a given  $\beta$ -LG variant or absence of relationship (*Amigo et al.*, 2000 ). However, there is an ongoing interest, especially in the Mediterranean countries where sheep are mainly kept for the production of cheese, concerning the relationship between the genetic variants of  $\beta$ -LG and traits related to milk and cheese production. Unfortunately milk constituents in the selection programmes of small ruminants have been taken into account to a lesser extent than it happened with cattle, due to several reasons including the difficulty of collecting a representative sample (*Dario et al.*, 2008).

*Curik et al.* (2002) studied genetic polymorphism of  $\beta$ -lactoglobulin gene with Pag ewes, belonging to 14 different flocks and located through the Pag Island (Croatia) and they reported two genetic variants (A and B) and three genotypes (AA, AB and BB) of  $\beta$ -lactoglobulin have been identified. According to the allele frequency (A=0.48, B=0.52) and occurrence of genetic variants, the Pag breed is similar to other Mediterranean dairy sheep breeds. The observed genotype frequencies at the  $\beta$ -lactoglobulin locus (AA=0.185, AB=0.589 and BB=0.226). They attributed excess of heterozygotes and allele frequencies at intermediate level to the superiority of the AB  $\beta$ -lactoglobulin genotype for milk production. Also, *Dario et al.* (2008) studied  $\beta$ -lactoglobulin gene polymorphism in Italian Leccese sheep and reported significant relationships with some milk components.

Barki sheep is well adaptive Egyptian breed to the harsh environmental condition in Egypt especially in semi arid region and this study aims to fingerprint of  $\beta$ -lactoglobulin gene in this native breed in an attempt to have clear image about genotypes frequencies of this gene and comparing with other Mediterranean dairy sheep breeds. This could be beneficial as the first step for genetic improvement of this well adaptive low milk producer breed and to draws more attention to these local genetic resources.

## Materials and Methods

### Animal materials and DNA extraction:

The present study was conducted on a total of 60 animals belonging to Barki sheep. Blood samples were taken randomly from sixty adult female ewes. The ewes are raised by farmers in North-west coast (Borg EL-Arab, Alexandria, Egypt) in which sheep are living in natural habitats. Approximately, 10ml 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 $\beta$ - LG gene:**

Allele discrimination was based on size differentiation of  $\beta$ - LG genetic markers. PCR conditions were performed according to *Feligini et al.* (1998). Primers LGB1 (5'- CAA CTC AAG GTC CCT CTC CA-3') and LGB2 (5'- CTT CAG CTC CTC CAC GTA CA-3') were used for amplification flanking a 120 base pair (bp) fragment, from the last 60 bp of intron 1 and exon 2 of ovine  $\beta$ -lactoglobulin gene (GenBank accession number X12817). The amplification was carried out using a pre-programmed thermal cycler (Eppendorf Mastercycler). After a first denaturation step at 95°C for 10 min, the samples were amplified for 34 cycles consisting of denaturation at 93°C for 15 s, primer annealing at 60 °C for 15 s, primer extension at 72 °C for 30 s followed by a 10 min final extension step at 72 °C.

Restriction enzyme digestion: A total volume of 15  $\mu$ L of each PCR product was digested with 10 U of *RsaI* endonuclease for 9 hours at 37°C. 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:**

Ovine DNA PCR amplification resulted in a 120 base-pair fragment of the  $\beta$ - lactoglobulin gene including exon 2. Restriction fragment length polymorphism was evidenced after digestion with *RsaI* endonuclease; in particular two restriction sites (GT/AC) for allele A and only one restriction site for allele B were detected. Allele discrimination was based on size differentiation (bp) of LGB; the three different genotypes, AA (66, 37 and 17 bp), AB (103, 66, 37 and 17 bp) and BB (103 and 17 bp) were detected for the Barki ewes.

## **Results and discussion**

Figure (1) represents agarose gel electrophoresis of PCR amplified  $\beta$  - LG gene (120 bp) of Barki sheep breed, while figure (2) represents different genotypes (AA, AB, BB) resulted from *RsaI* digested  $\beta$  - LG gene product of this breed.

Genotyping at the  $\beta$  - LG locus at the DNA level revealed the presence of two alleles (A, B). In particular two restriction sites (GT/AC) for allele A and only one restriction site for allele B were detected. Allele discrimination was based on size differentiation (bp) of LGB; the three different genotypes, AA (66, 37 and 17 bp), AB (103, 66, 37 and 17 bp) and BB (103 and 17 bp) were detected for the Barki ewes. In Fig. 2, 17 bp fragment is very weak or not visible on the gel.

The frequencies of AA, AB and BB genotypes in Barki sheep breed were 0.45, 0.30, and 0.25. The allelic frequencies (A, B) are 0.60, 0.40 for this breed, respectively.

It is obvious from these findings that AA genotype has the highest frequency (0.45), while AB and BB genotypes were almost similar and lower than AA genotype 0.30, 0.25, respectively. This results differ from what obtained by *Curik et al.* (2002) as they observed genotype frequencies at the  $\beta$ - lactoglobulin locus (AA=0.185, AB=0.589 and BB=0.226). The main differences between our data and this finding are that heterozygote genotype (AB) frequency is the highest and more than what obtained in the present study. They mentioned that gene frequency and the occurrence of genetic variants the Pag sheep is similar to other Mediterranean dairy sheep breeds where alleles A and B of  $\beta$ -LG locus occur at the intermediate allele frequency. Another difference in the present data is that the majority of populations, the B allele is more frequent than A. The slight exceptions which agree with our data are two Spanish (Manchega and Segureña) and two Italian (Barbaresca-siciliana and Massese) breeds where A variant is the most frequent one.

The results obtained in the present study are partially agree with the findings of *Dario et al.* (2008) in Italian Leccese sheep as they reported that frequency of AA genotype (0.375) is more than BB (0.125), while as the majority of Mediterranean dairy sheep breeds heterozygote genotype(AB) frequency is the highest one (0.50). Also they reported that Milk composition did not differ considerably among the LGB genotypes, the main differences were found for fat content ( $P > 0.01$ ), with AA=AB>BB genotype, and whey protein content ( $P > 0.05$ ) with BB=AB>AA genotype and the polymorphism of  $\beta$ -LG locus in Leccese sheep is comparable to the results for some dairy and meat type ewes described in literature.

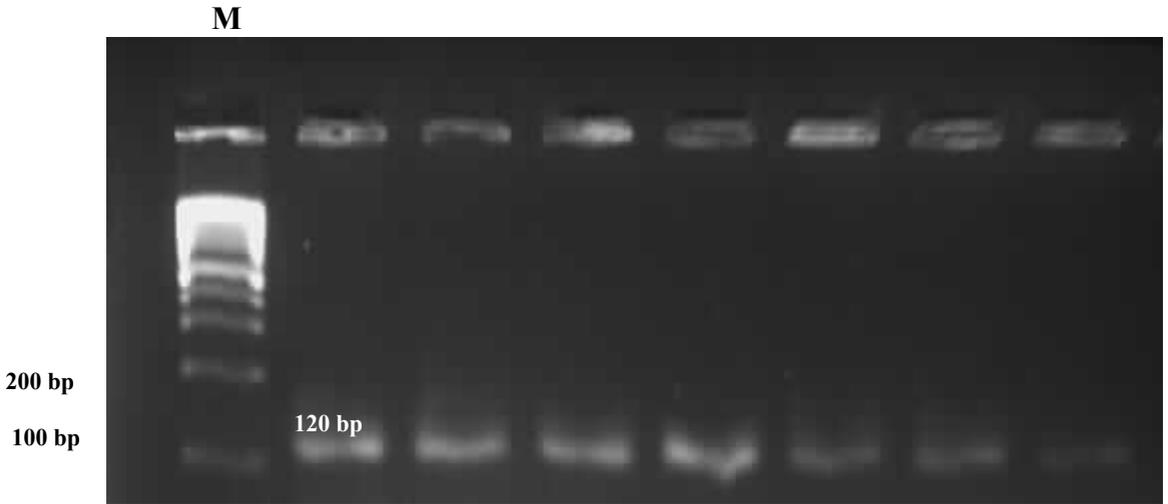


Figure 1. PCR amplification of  $\beta$  - LG gene (120 bp, Lanes 2-8) in Barki sheep breeds . Lane M, molecular size marker (100 bp DNA ladder).

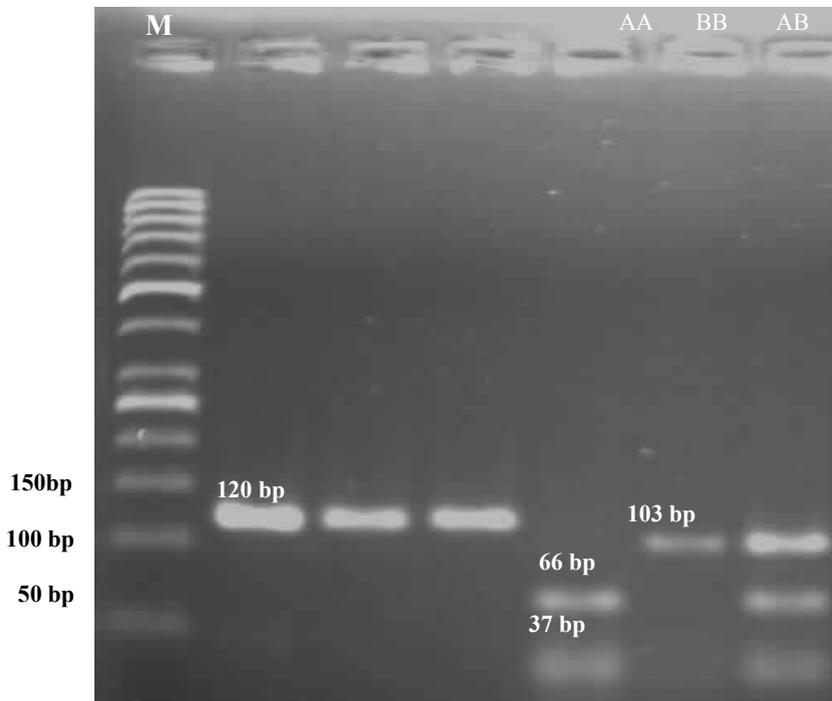


Figure 2. Agarose gel (3 %) shows the results of PCR (120 bp, Lanes 2-4) and after digestion by *RsaI* of animals with AA, AB, BB genotypes (Lanes 5-7). Lane M, molecular size marker (50 bp DNA ladder).

*Curik et al.* (2002) explained the reason that two genetic variants of the  $\beta$ -LG (A and B) are maintained at the intermediate gene frequency in the Mediterranean dairy sheep populations on the basis that continuous weak selection performed by farmers and potential superiority of the AB  $\beta$ -LG genotype for milk and/or cheese production. The observed excess of heterozygotes in the other studies suggests, also, the superiority of the AB  $\beta$ -LG genotype for milk and/or cheese production. On the other hand the low AB genotype frequency observed in the present study may be due to the low milk production of Barki ewes and the absence of selection to milk and/or cheese production traits in this breed. Further studies using large number of animals from different geographic regions should be made refer the relationship between  $\beta$ -LG genetic variants and traits related to milk and cheese production in Barki and other Egyptian sheep breeds.

## Conclusion

A total of 60 individuals females belonging to Barki sheep breed were analyzed for  $\beta$ -LG polymorphism at DNA level by the PCR-RFLP method. The amplified product was observed as 120 bp and the restriction digestion with *RsaI* revealed three genotypes, namely AA, AB and BB at the  $\beta$ -LG locus. The frequencies of AA, AB and BB genotypes in Barki sheep breed were 0.45, 0.30, and 0.25. The allelic frequencies (A, B) are 0.60, 0.40 for this breed, respectively.

Further researches are likely to be performed in order to provide more information on the current state of in situ conservation of animal genetic resources. This would open promising perspectives in the improvement of selection breeding programmes accuracy and intensity to enable local breeders guarantee the safeguard of local breeds.

## Polimorfizam $\beta$ - lakto globulin gena kod ovaca rase Barki

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## Rezime

Polimorfizam  $\beta$  - LG gena na nivou DNK je analiziran korišćenjem PCR-RFLP I dobijene su dve genetske varijante  $\beta$ -LG gena u kod ovaca autohtone Pag i

Leccese rase. Međutim, nijedno istraživanje do sada se nije bavilo lokalnom egipatskom rasom ovaca Barki u vezi sa polimorfizmom  $\beta$ -LG na nivou DNK. Veza između  $\beta$ -LG polimorfizma i proizvodnje mleka, kao i prerađivačkim odlikama sira dobijenog od mleka ovaca je ispitivana u ovoj studiji. Međutim, rezultati su oprečni, i ukazuju s jedne strane na superiornost date  $\beta$ -LG varijante ili odsustvo odnosa. Metoda „fingerprinting“  $\beta$ -LG gena i odnosa još uvek nije pojašnjenja kod lokalne rase Barki, kao i drugih rasa ovaca koje se gaje u Egiptu. U vezi sa značajem Barki rase ovaca kao lokalne adaptivne rase koja se gaji u Egiptu, ova preliminarna studija koja je imala za cilj izdvajanje  $\beta$ -LG gena kod ovaca rase Barki, u pokušaju da se dobije jasnija slika o genotipovima  $\beta$ -LG gena kod ove rase. Ukupno 60 ovaca rase Barki je bilo uključeno u ispitivanje metodom PCR-RFLP. Ovece su odgajane na farmama severno-zapadne obale (Borg EL-Arab, Aleksandrija, Egipat) u prirodnim staništima. Amplifikovani proizvod je posmatran kao 120 bp a restriktivna digestija sa *Rsa* I otkrila je tri genotipa, naime AA, AB i BB na  $\beta$ -LG lokusu. Frekvencije AA, AB i BB genotipova su bile 0.45, 0.30, i 0.25, respektivno. Potrebna su dalja istraživanja kako bi se dobilo više informacija o trenutnom stanju in situ očuvanja životinjskih genetskih resursa. To bi otvorilo perspektive u smislu poboljšanja tačnosti selekcije i odgajivačkih programa i intenziteta da se obezbedi odn. garantuje loklanim odgajivačima očuvanje lokalnih rasa..

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