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Polymorphism of β-Lactoglobulin Gene in Iranian Sheep Breeds Using PCR-RFLP

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Abstract: β-lactoglobulin is the major milk whey protein in ruminants and its coding gene located on ovine chromosome 3. This protein produces in mammary glands during pregnancy and lactation stages. Studies have shown that the protein is polymorphic in many breeds of sheep. This is the result of single base pair substitution in β-lactoglobulin gene that also rises to RsaI restriction Fragment Length Polymorphism (RFLP). Blood samples were supplied from 142 animals of Iranian sheep breeds (Ghezel, Afshary, Moghani, Makoii and Arkharmerino). Genomic DNA was extracted from 100 μL blood sample according to Boom method modified by Shaikhayev. Gel monitoring and spectrophotometeric methods were used for determination of DNA quality and quantity. BLg 5 and 3 primers amplified a 452 bp fragment from exon II of ovine β-lactoglobulin gene. Products of amplification were recognized by electrophoresis on 1% agarose gel stained with ethidium bromide. RsaI enzyme was used for restriction of PCR products. Digestion products were separated by electrophoresis on 8% non-denaturant polyacrylamide gel and visualized after staining with ethidium bromide on UV gel documentation. PopGen 32 software (ver. 1.31) was used to estimating the frequency of allele, genotype and Hardy-Weinberg equilibrium. Frequency of A-allele in Ghezel, Afshary, Moghani, Makoii and Arkharmerino sheep was 56, 34, 36, 53 and 48%, respectively. The populations were in Hardy-Weinberg equilibrium except of Afshary breed.

Key words: β-lactoglobulin, Iranian sheep breeds, PCR-RFLP, polymorphism, population, equilibrium

INTRODUCTION

Polymorphisms of milk proteins can be used as marker of systems. Since the initial discovery of milk protein polymorphisms in cow's milk, extensive research has revealed additional polymorphic systems in α-lactalbumin, β-lactoglobulin as well as in different caseins. β-lactoglobulin is the major milk whey protein in ruminant's milk. β-lactoglobulin has been the subject of many physicochemical studies and the studies show that this protein is polymorphic in many breeds of sheep. Research showed that the ovine BLg transcription units are 4.9 kb long, coprising seven exons and six introns. In ruminants BLg consists of a mature polypeptide chain of 162 amino acids which forms a stable dimer in milk. Until now three genetic variants have been found in sheep: A, B and C (Anton *et al*., 1999). The amino acid sequencing has shown that a Tyr^{20} in Blg-A is replaced by a His in BLg-B (Kolde and Braunitzer, 1983). This is the result of single base pair substitution in the β-lactoglobulin gene which also rises to an RsaI restriction fragment length polymorphism. Sequence information from alleles A and B (Ali *et al*., 1990) reveals a RsaI restriction site in allele A but not in allele B. Amino acid 20 in allele A is Tyr that encoded by a TAC codon. In allele B codon CAC in same position is responsible for His. The variant C is a subtype of variant A with a single amino acid exchange of Arg→Glu at position 148 (Erhardt, 1989). Polymerase chain reaction (Saiki *et al*., 1988) can use to amplify the polymorphic region of the gene. As β-lactoglobulin is known to bind a variety of hydrophobic molecules including retinol, a function in transport of vitamin A has been proposed (Papiz *et al*., 1986).

The genotype BB of β-lactoglobulin seems to be associated with higher milk yield. On the other hand genotypes AA and AB seem to be superior in protein and casein content of milk, crude protein yield and cheese making properties (Garzon and Martinez, 1992). B allele of BLg is associated with higher milk yield than allele A in a flock of 207 Sardinian ewes (Bolla *et al*., 1989). No data is available concerning the relationship between the BLg-C allele and production traits or milk properties. The aim of the present study was identification of two genetic variants (A and B) and three genotypes (AA, AB and BB) of BLg gene in Iranian sheep breeds by PCR-RFLP.

MATERIALS AND METHODS

Blood samples were supplied from 142 sheep of Iranian breeds (Ghezel, Afshary, Moghani, Makoii and

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Arkharmerino). In order to prevention of blood coagulation, EDTA solution (0.5M, pH 8) was added to samples.

DNA was extracted from 100 μL blood according to Boom *et al.* (1989) method modified by Shaikhayev (1995). Gel monitoring and spectrophotometeric methods were used for determination of DNA quality and quantity. Yield of DNA per preparation were about 1OD from 100 μL of blood. For polymorphic region amplification of BLg gene, 3 μL DNA solution (50-100 ng) was added in a total volume of 25 μL PCR mix. Biometra thermocycler, UNIOII model was used for amplification.

The PCR mix contained 2.5 μL PCR buffer (670 mM Tris-HCl pH 8.8, 160 mM $(NH4)_2SO_4$, 0.1% mM Tween 20), 1.5 μL $MgCl_2$, 2.5 μL each dNTPs (2mM from each nucleotide), 2 μL mix of oligonucleotides (50 ng from each primer), 0.3 μL Taq DNA polymerase (5 u μL^{-1}) and 10 μL ddH_2O. Samples were amplified for 35 cycles at the following regime: denaturation step at 95°C for 1 min, primer annealing at 65°C for 30 sec and extension step at 74°C for 40 sec. Primers BLg5 (5'-TTG GGT TCA GTG TGA GTC TGG-3') and BLg3 (5'-AAA AGC CCT GGG TGG GCA GC-3') amplified a 452 bp fragment from exon II of ovine β-lactoglobulin gene (Eignatev, 1998). Products of amplification were recognized by electrophoresis on 1% agarose gel stained with ethidium bromide.

About 8 μL of each PCR reaction samples were incubated for 3 h at 37°C with 2 μL (2 u μL^{-1}) RsaI enzyme. Digestion products were separated by electrophoresis on 8% non-denaturant polyacrylamide gel and visualized after staining with ethidium bromide on UV gel documentation. PopGen32 software (ver. 1.31) was used to estimating the frequency of allele, genotype and Hardy-Weinberg equilibrium (Yeh and Yang, 2000). X^2 analysis was performed for each breed to test the goodness of fit to Hardy-Weinberg equilibrium expectations for the distribution of β-lactoglobulin phenotypes.

RESULTS AND DISCUSSION

DNA extraction produced high quality and quantity of genomic DNA that shown in Fig. 1. A 452 bp fragment of the ovine β-lactoglobulin gene from exon II was amplified and has not any nun-specific bands (Fig. 2). After PCR amplification, enzymatic digestion and gel electrophoresis, DNA from AA homozygotes shows four bands of 175, 170, 66 and 41 bp. BB homozygotes give three bands of 236, 175 and 41 bp and heterozygotes have all five distinct bands (Fig. 3).

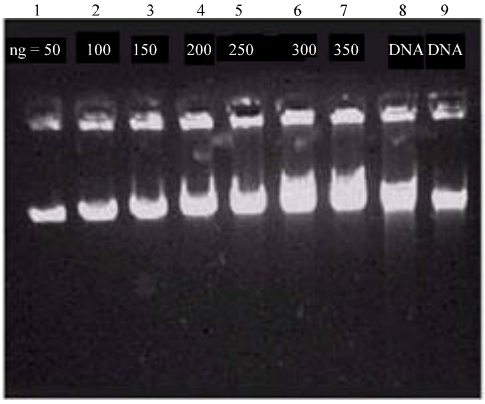

Fig. 1: DNA extracted from sheep blood samples on 1% agarose gel: lanes 1-7 are variable amount of λDNA (ng); lanes 8 and 9 are extracted DNA samples

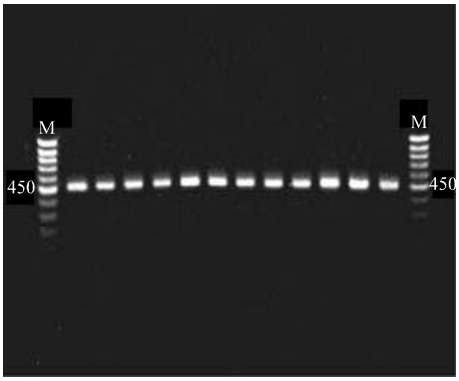

Fig. 2: Ethidium bromide stained agarose gel of PCR products from exon II of ovine BLg gene: lanes M are 100 bp DNA size marker and other lanes are PCR products

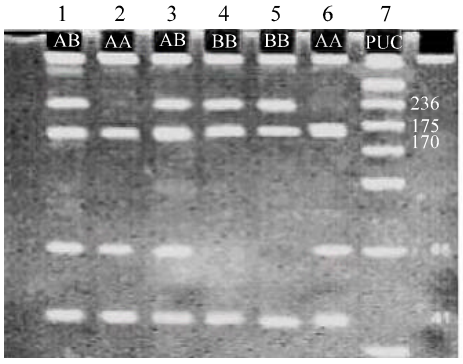

Fig. 3: RFLP analysis of 452 bp fragment of exon II of ovine BLg gene by RsaI enzyme on 8% polyacrylamide gel: genotypes AA (lanes 2 and 6), AB (lanes 1 and 3) and BB (lanes 4 and 5) are shown. Lane 7 is PUC19 size marker

Table 1: Distribution of β-Lactoglobulin phenotype and allele frequencies in Iranian sheep breeds

	Genotype number			Genotype frequency			Allele frequency		
Sheep breeds	AA	AB	BB	AA	AB	BB	A	B	X^2
Ghezel	10	16	6	0.31	0.50	0.19	0.56	0.44	0.000
Arkharmerino	4	1	25	0.19	0.57	0.24	0.48	0.52	0.308
Makoii	8	18	6	0.27	0.53	0.20	0.53	0.47	0.089
Moghani	4	13	12	0.14	0.45	0.41	0.36	0.64	0.065
Afshary	1	18	10	0.03	0.62	0.35	0.34	0.66	3.708*

The distributions of β-lactoglobulin genotypes of Iranian sheep are shown on Table 1. In total tested sample bout 2 alleles were identified: A and B that control the occurrence of 3 phenotypes: AA, AB and BB. The highest values of AA, AB and BB genotype frequency were found in Ghezel (0.31), Afshary (0.62) and Moghani (0.41), respectively. The populations were in Hardy-Weinberg equilibrium except Afshary breed. The highest value of A allele was found in Ghezel sheep. There was a good agreement between the observed frequencies and those expected on the basis of the Hardy-Weinberg law in each of the demonstrated breeds. Within all breeds β-lactoglobulin A and B variants have been observed.

The studies undertaken provided information on the polymorphism of ovine β-lactoglobulin in Iranian sheep breeds. The frequency of AB genotype in the studied breeds in this investigation was highest than other genotypes. Recently similar results for AB genotype of ovine β-lactoglobulin in pag ewes (Croatia) are reported by Cubric-Curik *et al.* (2002). Anton *et al.* (1998) reported positive results. Nassiry *et al.* (2002) didn't find BB genotype in Russian karakol sheep breeds.

Di Stasio *et al.* (1997) reported that frequency of allele A and B in Valle del belice breed were 0.35 and 0.65, respectively. Also the frequency of allele A 0.58 and allele B 0.41 was reported (Saiki *et al.*, 1988).

The ovine β-lactoglobulin types A and B observed in this survey have been reported in sheep by electrophoretic patterns of proteins. However, only few previous researchers examined the PCR-RFLP method for ovine β-lactoglobulin. The results of this study provided more information on the polymorphism of ovine β-lactoglobulin (a genetic marker).

CONCLUSION

These data provide evidence that Iranian sheep breeds have a variability which opens interesting prospects for future selection programs especially marker assisted selection between different genotypes of milk and cheese characteristics and also for preservation strategies. Finally data show that PCR-RFLP is an appropriate tool for evaluating genetic variability.

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