aJava

Asian Journal of Animal and Veterinary Advances

Academic
Journals Inc.

www.academicjournals.com

Asian Journal of Animal and Veterinary Advances, 4 (3): 130-133, 2009
ISSN 1683-9919

Allelic Frequencies of a *Sac*II RFLP at Exon 7 of the β-lactoglobulin Gene in Turkish Hair Goat Breed

C. Elmaci, Y. Oner and M. Koyuncu
Department of Animal Science, Faculty of Agriculture, Uludag University, Bursa, Turkey

Abstract: Polymorphism in the exon 7 to the 3' flanking region of β-lactoglobulin (β-lg) gene in Turkish hair goat populations were investigated. The study was carried out including 233 hair goats using PCR-RFLP. Digestion of amplification product with *Sac*II restriction enzyme revealed two alleles namely S_1 and S_2 (which was produced by a single nucleotide substitution) and three genotypes (S_1S_1, S_1S_2 and S_2S_2) in the studied population. The genotypic frequencies of S_1S_1 and S_1S_2 were almost eqnal. S_2S_2 genotype was found to be lower than other genotypes (S_1S_1 and S_1S_2) in the studied population. The allele frequencies of S_1 and S_2 at β-lg locus were 0.67 and 0.33 in hair goat population, respectively. Deviation from Hardy-Weinberg equilibrium was not detected.

Key words: β-lactoglobulin, goat, polymorphism, PCR-RFLP

INTRODUCTION

The first report of genetic polymorphism of bovine β-lactoglobulin (β-lg) was reported by Aschaffenburg and Drewry (1955). Since, then genetic polymorphism of milk proteins has been of considerable interest in animal breeding and in the dairy industry and possible relationships between milk protein polymorphism and production traits, milk composition and quality have been widely studied by Pagnacco and Caroli (1987), Ng-Kwai-Hang *et al*. (1990) and Baranyi *et al*. (1993). β-lg is a major whey protein in the milk of ruminants. It is also found in the milk of most mammals with exception of man, rodents and lagomorphs (Perez and Calvo, 1995). Until now, several variants of bovine and ovine β-lg have been described both at protein level and DNA level (Gaye *et al*., 1986; Erhardt, 1989; Elmaci *et al*., 2006). The two genetic variants (A and B) of β-lg were reported in goat milk (Moioli *et al*., 1998) at protein level. However, no variants resulting in amino acid substitution have been identified at DNA level in goat but Pena *et al*. (2000) and Yahyaoui *et al*. (2000) have described polymorphism in exon 7 and in the β-lg proximal promoter region of Spanish and French goats. β-lg gene polymorphism at the DNA level has been analyzed by PCR-RFLP (Pena *et al*., 2000) and they also reported two novel genetic variants of the β-lg gene in goat breeds. β-lg polymorphism was reported in different goat populations at protein level in Turkey (Ozdil and Asal, 2002; Turkyilmaz, 2003; Gurcan, 2005) and two alleles, $\beta\text{-lg}^A$ and $\beta\text{-lg}^B$, at β-lg locus in the studied goat populations were reported. However, no information has been reported in Turkish goats regarding β-lg polymorphism at the DNA level. Therefore, the aim of present research was to study the genetic polymorphism of the β-lg gene (exon 7) at DNA level in Turkish hair goat using PCR-RFLP method.

MATERIALS AND METHODS

Animal Resources and DNA Isolation

Hair goats are raised in all parts of Turkey, particularly in mountainous and bushy areas of Mediterranean. Hair goat is also called ordinary goat or black goat. It is major goat breed of Turkey.

Corresponding Author: Cengiz Elmaci, Department of Animal Science, Faculty of Agriculture, Uludag University, Bursa, Turkey Tel: 90 224 2941554 Fax: 90 224 4428152

Blood samples were collected randomly from a total of 233 hair goats belonging to four different flocks around Bursa region in their natural habitats. An effort was made to collect samples from unrelated individuals based on information provided by farmers. Genomic DNA was isolated using Genomic DNA isolation kit following the manufacturer's protocol. The quality of DNA was checked on 0.8% agarose gels and concentrations were measured by UV-spectrophotometer.

Amplification of β-lg Gene and Restriction Enzyme Digestion

PCR amplifications of the β-lg gene were performed in a 25 μL reaction volume containing 50-100 ng genomic DNA, 2X PCR Master Mix and 0.5 μM each of the primers, Forward 5'-CGGGAGCCTTGGCCCTCTGG-3' and reverse 5'-CCTTTGTCGAGTTTGGGTGT -3', (Pena *et al*., 2000). The amplification reactions were performed in an Techgene Thermal Cycler (Techne, Cambridge, UK) programmed for an initial denaturation at 95°C for 5 min, followed by 35 cycles each with denaturing at 95°C for 30 sec, annealing at 65°C for 1 min, extension at 72°C for 90 sec and a fiual extension at 72°C for 5 min. The products were visualized by staining with ethidium bromide after electrophoresis of 10 μL reaction mixture on a 2 % agarose gel.

PCR products (12 μL) were digested with 10 U of the *Sac*II (Fermentas) restriction enzyme in a 20 μL total reaction volume overnight at 37°C. The restriction fragments were directly analyzed by electrophoresis in 3% agarose gels in 1X TAE bnffer stained with ethidium bromide and visualized under UV light.

Statistical Analysis

Direct counting was used to estimate genotypic and allelic frequencies of β-lg genetic variants. The chi-square test (χ^2) was used to check whether the populations were in Hardy-Weinberg equilibrium. All calculations and the χ^2 analysis were carried out using PopGene32 software (Yeh *et al*., 2000).

RESULTS AND DISCUSSIONS

β-lg polymorphism was reported in different goat populations at protein level in Turkey. Ozdil and Asal (2002), Turkyilmaz (2003) and Gurcan (2005) described two allele, $\beta\text{-lg}^A$ and $\beta\text{-lg}^B$, at β-lg locus in different goat breed populations in Turkey. In Turkish hair goat population, $\beta\text{-lg}^B$ allele were found to be predominant at protein level (Ozdil and Asal, 2002; Gurcan, 2005). However, no information has been reported in Turkish goats regarding β-lg polymorphism at the DNA level.

The exon 7 to the 3' flanking region of β-lg gene of Turkish hair goat were investigated. A fragment of 426 bp from exon 7 to the 3' flanking region was successfully amplified by PCR and digested with *Sac*II restriction enzymes to detect the presence of S_1 or S_2 variants. Digestion with *Sac*II restriction enzyme reveals the polymorphic sites, which were produced by a single nucleotide substitution in position of +4601. Allele discrimination was based on size differentiation of digested fragments. Restriction digestion of 426 bp PCR products with *Sac*II enzymes revealed three genotypes (Fig. 1) of S_1S_1 (349 and 77 bp), S_1S_2 (426, 349 and 77 bp) and S_2S_2 (426 bp). Screening for allele frequency at β-lg locus at DNA level in goat population revealed the presence of two alleles, namely S_1 and S_2.

Table 1 shows the allelic and genotypic frequencies in investigated goat population. The genotypic frequencies of S_1S_1 and S_1S_2 were almost equal. S_2S_2 genotype was found to be lower than other genotypes (S_1S_1 and S_1S_2) in the studied population. The frequency of S_2 allele at β-lg locus was found to be lower than that of S_1 allele. It means that, the frequency of S_1 allele was clearly predominant. The chi-square test results are shown in Table 1 and revealed that population was in Hardy-Weinberg equilibrium.

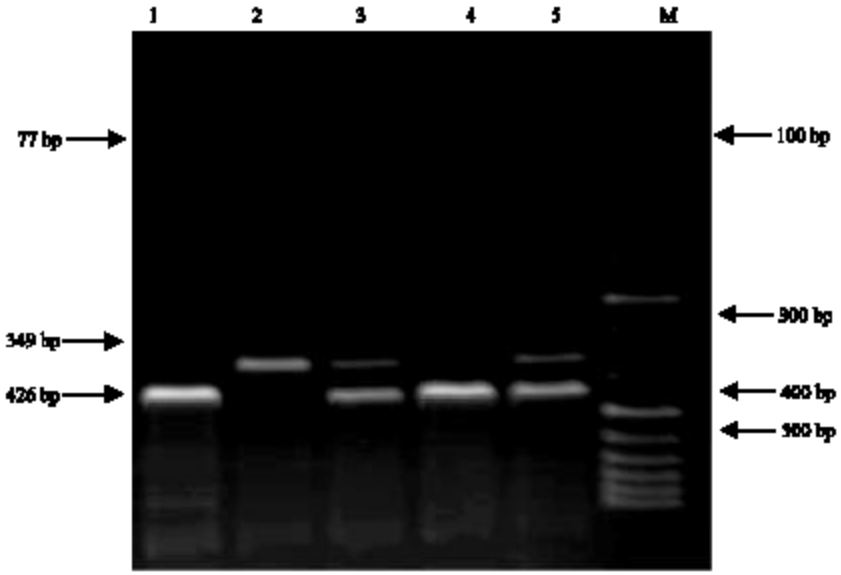

Fig. 1: Electrophoresis of exon 7 of goat β-lg gene amplified by PCR of animal with S_1S_1, S_1S_2 and S_2S_2 variants and digested with *Sac*II. The 426 bp band was restricted into two bands 349 and 77 bp in the S_1 polymorphism (Lane 1 and 4: S_2S_2; Lane 2: S_1S_1; Lane 3 and 5: S_1S_2; M: marker)

Table 1: Gene and genotypic frequencies of β-lg locus in the studied Turkish hair goat population

	Gene frequency		Genotypic frequency			
N	S_1	S_2	S_1S_1	S_1S_2	S_2S_2	Chi-square test
233	0.67	0.33	0.45	0.44	0.11	0.155^{NS}

^{NS}: Non significant

Polymorphism studies conducted in some Indian breeds (Kumar *et al*., 2006) showed higher frequencies of S_2 allele than the present study. Kumar *et al*. (2000) showed that the frequency of S_2S_2 genotype was more frequent than other genotypes (percentage of S_2S_2 genotype varied from 41.7% to 100%) and S_2 allele frequency was found to be the higher than S_1 allele frequency. The frequencies of S_1 and S_2 allele in this study found to be similar to French and Spanish goat breeds studied previously by Pena *et al*. (2000). Consistent with the result of this study, an S_1 allele frequency in French and Spanish goat breeds was higher than S_2 allele frequency. On the other hand, some polymorphic sites were reported different region of goat β-lg gene. Graziano *et al*. (2003) and Ballester *et al*. (2005) described different polymorphism in proximal promoter region and in exons 1, 2, 3 and 6 of the goat β-lg gene in different goat breeds.

CONCLUSION

In the present study, we demonstrated that there was a genetic polymorphism in samples collected from Turkish hair goat breed. Milk protein polymorphism has been considered a potential tool for selection of goat breeds. The results of this study showed that Turkish hair goat breed have a genetic variability in the β-lg locus, which may lead to extensive study of genetic polymorphism of milk proteins in Turkish hair goat breed. This record may be useful for marker-assisted selection between different genotypes and the technological properties of milk. But, it is necessary to screen further goat breeds for determining the polymorphism at proximal promoter region of β-lg gene.

ACKNOWLEDGMENT

This study was supported by grants from the Scientific Research Projects Council of Uludag University (Project no: Z-2006/37), Bursa, Turkey.

REFERENCES

Aschaffenburg, R. and J. Drewry, 1955. Occurrence of different β-lactoglobulins in cow's milk. Nature, 176: 218-219.

Ballester, M., A. Sanchez and J.M. Folch, 2005. Polymorphism in goat β-lactoglobulin gene. J. Dairy Res., 72: 379-384.

Baranyi, M., Z.S. Bozse, J. Buchberger and I. Krause, 1993. Genetic polymorphism of milk proteins in *Hungarian spotted* and *Hungarian grey* cattle: A possible new genetic variants of β-lactoglobulin. J. Dairy Sci., 76: 630-636.

Elmaci, C., Y. Oner and M.S. Balcioglu, 2006. Genetic polymorphism of β-lactoglobulin gene in native Turkish sheep breeds. Biochem. Genet., 44: 379-384.

Erhardt, G., 1989. Evidence for a third allele at the β-lactoglobulin (β-Lg) locus of sheep and its occurrence in different breeds. Anim. Genet., 20: 197-204.

Gaye, P., D. Hue-Delhaie, J.C. Mercier, S. Soulier, J.L. Vilotte and J.P. Furet, 1986. Ovine β-lactoglobulin messenger RNA: Nucleotide sequence and mRNA levels during functional differentiation of the mammary gland. Biochemie, 68: 1097-1107.

Graziano, M., M. D'Andrea, A. Angiolillo, R. Lagonigro and F. Pilla, 2003. A new polymorphism in goat β-lactoglobulin promoter region. Italian J. Anim. Sci., 2: 67-70.

Gurcan, N., 2005. The polymorphism of β-lactoglobulin in some Angora and hair goat populations. The polymorphism of β-lactoglobulin in some Angora and hair goat populations. M.Sc. Thesis, Ankara University, Graduate School of Natural and Applied Science, Ankara, Turkey.

Kumar, A., P.K. Rout and R. Roy, 2006. Polymorphism of at the β-lactoglobulin gene in Indian goats and its effect on milk yield. J. Applied Genet., 47: 49-53.

Moioli, B., F. Pilla and C. Tripaldi, 1998. Detection of milk protein genetic polymorphism in order to improve dairy traits in sheep and goats: A review. Small Rumin. Res., 27: 185-195.

Ng-Kwai-Hang, K.F., H.G. Monardes and J.F. Hayes, 1990. Association between genetic polymorphism of milk proteins and production traits during three lactations. J. Dairy Sci., 73: 3414-3420.

Ozdil, F. and S. Asal, 2002. The polymorphism of β-lactoglobulin. Proceedings of the 3rd Annual Meeting on Animal Science, Sept. 14-16, 2002, Ankara, Turkey.

Pagnacco, G. and A. Caroli, 1987. Effect of casein and β-lactoglobulin genotypes on renneting properties of milks. J. Dairy Res., 54: 479-485.

Pena, R.N., A. Sanchez and J.M. Folch, 2000. Characterization of genetic polymorphism in goat β-lactoglobulin gene. J. Dairy Res., 67: 217-224.

Perez, M.D. and M. Calvo, 1995. Interaction of β-lactoglobulin with retional and fatty acids and its role as a possible biological function fort his protein: A review. J. Dairy Sci., 78: 978-988.

Turkyilmaz, O., 2003. The milk protein polymorphism in Saanen goats with high milk yielding. Ph.D Thesis, Uludag University, Institute of Health Sciences, Bursa, Turkey.

Yahyaoui, M.H., R.N. Pena, A. Sanchez and J.M. Folch, 2000. Rapid communication: Polymorphism in the goat β-lactoglobulin proximal promoter region. J. Anim. Sci., 78: 1100-1101.

Yeh, F., R.C. Yang and T. Boyle, 2000. Popgene (v.1.32), microsoft windows-based freeware for population genetic analysis. http://www.nalberta.ca/~fyeh/Pop32.exe.