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Genetic Polymorphisms of α-lactalbumin and β-lactoglobulin in South Anatolian and East Anatolian Red Cattle

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Abstract: The objective of the present study was to determine the genotype and allele frequencies for alpha-lactalbumin (α-LA) ve β-lactoglobulin (β-LG) that are claimed to be associated with milk production traits in cattle in South Anatolian Red (SAR) and East Anatolian Red (EAR) cattle. In this study, 40 cattle for each of SAR and EAR were used. Genomic DNA samples were isolated by using standard salt-out method. After Polymerase Chain Reaction (PCR), α-LA and β-LG genes were digested with *Msp*I and *Rsa*I (R5), *Ava*I (R3), *Msp*I (R1), *Sau*3A (R2) restriction enzymes, respectively. As a result, SAR and EAR cattle breeds have the lower allel frequencies for α-LA and β-LG gene than high-yielding European dairy cattle breeds. Because of that reason we may claim that applying the selection programs for developing the allels belonging to both genes may contribute to the trials to improve the production parameters in SAR and EAR breed bovines.

Key words: SAR, EAR, genetic polymorphism, α-LA, β-LG

INTRODUCTION

A number of studies have been performed on milk proteins since Aschaffenburg and Drewy (1955) demonstrated the A and B variants of β-LG in cow milk in 1955. Each of milk proteins that have been determined basing on homology of their structures (Farrell *et al*., 2004). Milk proteins follow condominant Mendel's law of inheritance. Many of these genes have been mapped and sequenced. Nowadays, polymorphisms of milk proteins can be determined at protein level and DNA level. Some of these polymorphisms in milk proteins are known to affect milk yield, milk composition, micelle organization, coagulation characteristics and cheese yield. Scientific investigations on polymorphisms of genes related to milk proteins usually focused on cow milk. According to Mendel's laws, 6 large milk protein fractions which are controlled by non-dominant autosomal genes exist at different allele forms in cattle; α_{s1} casein, α_{s2} casein, β casein, $_{K}$ casein, α-LA and β-LG. Four casein variants are encoded by a cluster of genes located on the 4th chromosome whereas those encoding α-LA and β-LG are located on the 5th and 11th chromosomes, respectively (Erhardt *et al*., 1997).

α-LA which is the least studied milk protein is required for lactose biosynthesis in mammary gland and therefore it plays an important role in milk production. α-LA has two genetic variants as A and B. Variant A differs form variant B because glutamine amino acid at the 10th position in variant B replaces with arginine. Such change at the 10th codone results in a Single Nucleotide Polymorphism (SNP) which can be recognized by MspI restriction in the presence of α-LA-B or in the lack of α-LA-A (Mitra *et al*., 1998). A lots of investigations shows that SNP in α-LA change the gene expression and deal with differences in milk yield and qnality (Ramesha *et al*., 2002).

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The gene encoding β-LG has been sequenced in sheep, cow and goat. It has been mapped on the 3rd chromosomes in sheep and on the 11th chromosome in goat and cattle. Although, biological function of β-LG which is the major milk serum protein in swine, horse, cat, whale, dolphins and ruminants is not entirely known yet, it has been thought that it may play a role in transport of retinol and fatty acids. β-LG is composed of 162 amino acids and is one of the major milk components. Its function has still not been known except for playing a role in transport of hydrophobic molecules such as retinol and small fatty acids (Godovac *et al*., 1985). It shows structural homologies with human retinol-binding protein and it has been speculated that it functions in vitamin A transport by binding retinol (Erhardt, 1989). In addition, β-LG has been reported to have a positive effect on digestion of milk proteins (Perez *et al*., 1992).

Some of the investigators indicated that synthesis of milk protein is not affected by enviroumental factors but affected by differences between A and B alleles which are produced by SNP in gene regions (Gelderman *et al*., 1996; Folch *et al*., 1999; Ford *et al*., 1993; Prosser *et al*., 2000)

The aim of this study is to determine the frequencies of alleles and genotypes of β-LG and α-LA genes which effect milk protein contents in SAR and EAR cattle.

MATERIALS AND METHODS

In the present study, unrelated 40 cattle for each of SAR and EAR breeds were used. SAR breed cattle were selected from the herds in South Anatolian region of Turkey (Diyarbakir, Hatay) and EAR cattle were selected from those located in Eastern Anatolian region of Turkey (Kars). Blood samples were collected in sterile 2 mL tubes containing EDTA. Genomic DNAs were isolated using a standard salt-out method (Miller *et al*., 1988). The PCR for α-LA and β-LG was carried out in a final volume of 25 µL containing 1 U Taq DNA polymerase (Fermentas Life Sciences, Canada), 2-2,5 µL 10×PCR buffer (750 mM Tris-HCl (pH 8.0), 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20, 1.5 mM $MgCl_2$, 50-100 ng genomic DNA, 100 µM dNTP (Takara, Biotechnology Co, Ltd, Japan) and 10 pmol of each primer. The primer sequence used for the β-LG; primer 1: AGCAACACACCCAGCACCAG and primer 2: CAAGCAGGAGGCACTTCATG (Wagner *et al*., 1994). Amplification program were 94°C for 3 min; 32 cycles of 94°C for 1.30 min, 58°C for 1.30 min and 72°C for 3 min and a final extension at 72°C for 5 min. Those samples that were positive at the end of PCR, the target region of β-LG gene which is a 854 bp sequence containing 795 bp of the promoter region and 59 bp of exon I is subjected to digestion by *Msp*I (Fermentas Life Sciences, Canada), *Sau*3AI, *Ava*I and *Rsa*I restriction enzymes. *Msp*I: allele A 730, 119, 5 and allele B 730, 89, 30 and 5 bp; *Sau*3AI: allele A 468, 386 and allele B 433, 386, 35; *Ava*I: allele A 734, 83, 37 and allele B 734, 120; *Rsa*I: allele A 588, 266 and allele B 854 bp. The digestion products were run through 2% agarose gel. The primer sequence used for the α-LA; primer 1: TTGGTTTTACTGGCCTCTCTTGTCATC and primer 2: TGAATTATGGGACAAAGCAAAATAGCAG (Mitra *et al*., 1998). Amplification program were 94°C for 5 min; 30 cycles of 95°C for 15 sec, 60°C for 30 sec, 72°C for 30 sec and a final extension at 72°C for 10 min. The amplified 309 bp is subjected to cleavage by *Msp*I enzyme and then the resulting products were separated by 2% agarose gel electrophoresis to differentiate the alleles B (220 and 89 bp) and AB (309, 220 and 89 bp).

Direct counting was used to estimate genotype and allele frequencies of α-LA, β-LG variants. The Chi-square test (χ^2) was used to determine whether the populations were in Hardy-Weinberg equilibrium using PopGene32 software (Yeh *et al*., 2000).

RESULTS

In both breeds, frequency of A alleles of R_2, R_3 and R_5 were found higher than that of B alleles. In both breeds, frequency of B alleles of R_1 was found higher than that of A alleles. BB genotypes of

R1 were found higher than the AA genotypes (Table 1). AA genotypes were found higher than the BB genotypes for R3. The PCR products of α-LA is shown in Fig. 1 and agarose gel separation of MspI PCR amplified products from α-LA AA, BB and AB is shown in Fig. 2. Genotype and allele frequencies determined in EAR and SAR cattle for MspI polymorphism of the α-LA are shown in Table 2. BB genotypes were found higher than the AA genotypes significiantly in both breeds. In both breeds, frequency of B alleles were found higher than that of A alleles.

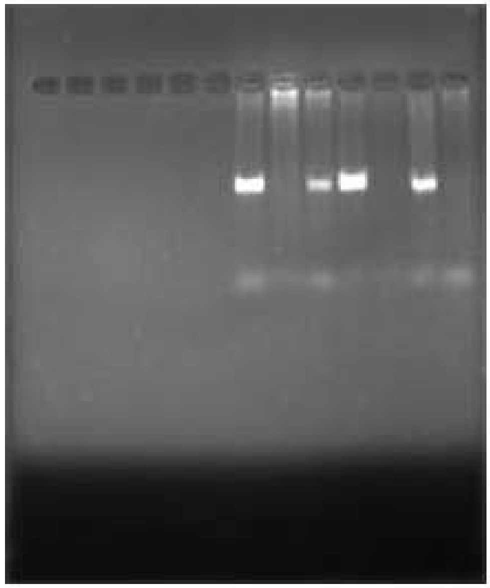

Fig. 1: PCR products for alpha-lactalbumin

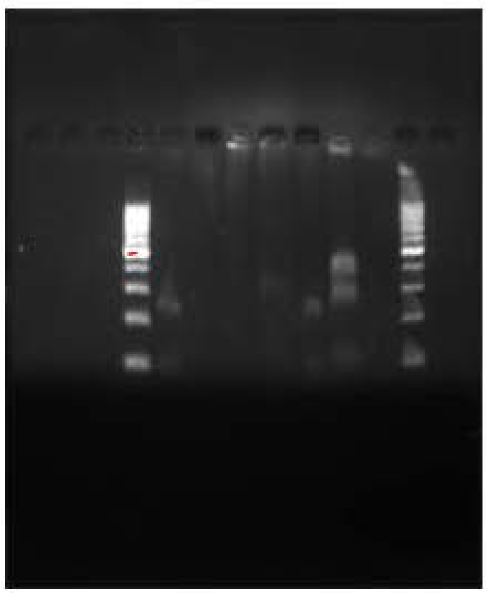

Fig. 2: Agarose gel seperation of *MspI*-digested PCR amplified products from alpha-lactalbumin

Table 1: Distribution of R_5, R_3, R_1, R_2 genotypes and allele frequencies in SAR and EAR cattle

			Allel frequency (%)		Genotype			
Locus	Breed	n	A	B	AA	AB	BB	$(\chi^2)^1$
R_5	SAR[2]	40	0.68	0.32	20	14	6	1.8617^{ns}
	EAR[3]	40	0.75	0.25	20	20	0	4.1864*
R_3	SAR	40	0.74	0.26	27	5	8	19.3294***
	EAR	40	0.53	0.47	20	2	18	33.3091***
R_1	SAR	40	0.40	0.60	16	0	24	41.1146***
	EAR	40	0.04	0.96	0	3	37	0.0399^{ns}
R_2	SAR	40	0.66	0.34	19	15	6	1.2198^{ns}
	EAR	40	0.48	0.52	7	24	9	1.4497^{ns}

[1]Test of Hardy-Weinberg equilibrium; ^{ns}: Not significant, [2]South anatolian red cattle, [3]East anatolian red cattle, *, **Indicate significant values

Table 2: Distribution of α-LA *Msp*I genotypes and allele frequencies in SAR and EAR

			Allel frequency (%)		Genotype			
Locus	Breed	n	A	B	AA	AB	BB	$(\chi^2)^1$
β-LG (*Msp*I)	SAR[2]	40	0.14	0.86	0	11	29	0.9143^{ns}
	EAR[3]	40	0.46	0.54	12	13	15	5.1570*

[1]Test of Hardy-Weinberg equilibrium; ^{ns}: Not significant, [2]South anatolian red cattle, [3]East anatolian red cattle, *, **Indicate significant values

DISCUSSION

Ehrmann *et al*. (1997) reported that the frequencies of variants A in the β-LG encoding gene were between 0.38-1.0 in Brown Swiss, Simmental, German Friesian and Jersey cattles. In a study by Gustafson and Lunden (2003), the frequency of allele A was higher compared to the others in Sweedish Red and White cattle which had higher milk fat concentration. In this study, the A allele frequencies assosiated with high milk fat concentration in SAR and EAR cattle were found between 0.52- 0.75. The frequency of allele A is known to be as high as 0.80-0.91 in high-yielding European dairy cattle breeds (Udina *et al*., 2001).

Some of the investigators reported that the β-LG genotype AA was found to be associated with high whey protein and milk-protein quantities (Lin *et al*., 1989; Mc Lean *et al*., 1984; Ng-Kwai-Hang *et al*., 1986, 1990). In present study, SAR and EAR those cattle with AA genotype relate to the high production tarits for R_5 polymorphism was higher than the other genotypes.

As for the influence of α-LA *Msp*I on the production traits of cows Mitra *et al*. (1998) reported that Zebu cattles (Sahiwal, Hariana, Tharparkar) with BB genotypes had higher milk yields compared to the other genotypes. Aschaffenburg (1968) reported that the frequency of α-LA A was higher in Indian Zebu (0.22-0.44) than in the most African Zebu (0.03-0.15). In this investigation the frequency of α-LA A was 0.14 in SAR cattles as like in the Zebu cattles. Anatolia is accepted as a primary centre of domestication for *Bos taurus* cattle and it is also widely accepted that considerable levels of *Bos indicus* introgression have occurred at this centre (Loftus *et al*., 1999; Kumar *et al*., 2003). The results found by Edwards (2007) which observed Zebu admixture in SAR and EAR cattle, also support this findings. In this study the frequencies for allele A for, which is characteristic for taurine breeds were estimated lower in SAR cattle than EAR cattle. It is possible that SAR cattle are genetically closer to Zebu cattle. This possibility is also supported by the phenotypic characteristics of SAR cattle, because some of the SAR cattle have a hump on their cidago region like Zebu cattle. Although, both of SAR and EAR cattles belong to the *Bos taurus*, they are similar to the *Bos indicus* in genetic position. So that the frequency of α-LA A alleles were found lower like Zebu cattles.

As a result, in both SAR and EAR cattles, the frequencies of A alleles of the β-LG gene which associated with milk protein quantities were lower than similiar to the high yielding European cattles. The frequencies of A alleles of the α-LA were found like Zebu cattles (*Bos indicus*). Because of that reason we may claim that applying the selection programs for developing the allels belonging to both genes may contribute to the trials to improve the production parameters in SAR and EAR breed bovines.

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