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Determination of Kappa Casein Gene Polymorphisms and Their Effects on Milk Composition in Some Native Cattle Breeds of Turkey

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Abstract: Polymorphisms in exon IV of bovine kappa-casein gene (*CSN3*) determine eleven allelic variants (A, B, C, E, F1, F2, G1, G2, H, I and J) for the gene. These variants are effective on milk production traits. The aim of this study was to detect kappa-casein polymorphisms in Anatolian Black (AB) and East Anatolian Red (EAR) native cattle breeds of Turkey. For this purpose, blood and milk samples were collected from Middle and East Anatolian region of Turkey. Milk samples were analyzed for protein, fat, lactose, solids, solids-not-fat content and density by using milk analyzer. After DNA was extracted from the blood samples, exon IV of *CSN3* gene was amplified by PCR. PCR products were digested with HindIII and HaeIII enzymes to distinguish allelic variants A, B and E. Genotype frequencies were determined as AA = 0.500, AB = 0.500 for Anatolian Black and AA = 0.567, AB = 0.433 for East Anatolian Red. Allele frequencies were determined as A = 0.750, B = 0.250 for Anatolian Black and A = 0.783, B = 0.217 for East Anatolian Red. The effects of genotypes on milk protein and SNF contents were found significant in EAR ($p<0.05$) and also found close to significant in AB ($p<0.1$). The association between CSN3 genotypes and milk composition suggests that CSN3 polymorphism might be a useful marker for genotypic selection such as marker assisted selection. Selection of B allele for CSN3 polymorphism could provide considerable economic advantage for increasing milk quality.

Key words: Native cattle breeds, kappa casein (CSN3), gene polymorphism, milk composition, enzymes

INTRODUCTION

The association of genetic polymorphism with milk production and composition has stimulated interest in using genetic polymorphism of casein genes in molecular Marker Assisted Selection (MAS) to improve milk performance traits in farm animals (Kumar *et al*., 2006). Kappa-casein plays an important role in the formation, stabilization and aggregation of the casein micelles thus, altering the manufacturing properties and digestibility of milk (Jann *et al*., 2004a). About 80% of milk protein is casein protein (Koczan *et al*., 1991) which is controlled by the casein gene family. They locate on bovine chromosome 6 at q31-q33 and composed with α_{s1}, α_{s2}, β and κ-casein genes (Mercier and Vilotte, 1993; Jann *et al*., 2004b). Until now, researchers have reported 11 genetic variants (A, B, C, E, F1, F2, G1, G2, H, I and J) in cattle for the κ-casein gene (Farrell *et al*., 2004). Kappa-casein protein is 169 amino acids long with varying regions at codons 136 and 148. The A variant has threonine (ACC) at codon 136 and aspartic acid (GAT) at codon 148 whereas the B variant has isoleucine (ATC) and alanine (GCT) at codons 136 and 148, respectively (Mercier *et al*., 1973).

Many research have reported that some of these bovine protein variants, particularly certain κ-casein are associated with milk production (Ng-Kwai-Hang *et al*., 1984; Bech and Kristiansen, 1990; Bovenhuis *et al*., 1992) and have a major influence on milk composition (Lunden *et a l*., 1997; Ng-Kwai-Hang, 1998; Robitaille *et al*., 2002) and its processing properties including cheese yield (Van den Berg *et al*., 1992; Lunden *et al*., 1997; Ng-Kwai-Hang, 1998).

In general, researchers believe that the κ-casein B variant is associated with higher fat, protein and casein in the milk and has a significant influence on cheese making properties of milk and superior rennet coagulation properties in comparison to AA or AB variants (Raj *et al*., 2008). The genotypes BB and AB are used in artificial insemination programs to obtain a

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greater increase of the frequency of these alleles in cattle populations of commercial interest (Otaviano *et al*., 2005).

The B allele is known to be associated with more desirable coagulation properties (Aaltonen and Antila, 1987; Davoli *et al*., 1990; Ikonen *et al*., 1997; Macheboeuf *et al*., 1993; Pagnacco and Caroli, 1987; Schaar, 1984; Tervala *et al*., 1985; Van den Berg *et al*., 1992) and protein composition of milk (Ikonen *et al*., 1997; Macheboeuf *et al*., 1993; Ng-Kwai-Hang *et al*., 1987; Schaar, 1984) than A allele. The E allele was associated with the poorest milk coagulation properties and lower protein content than B and A alleles (Ikonen *et al*., 1999a).

Anatolian Black and East Anatolian Red breeds raised in Middle and East Anatolia region of Turkey, respectively. These breeds are adaptable for all climates, strong for bad conditions and feding, resistance to diseases.

Anatolian Black and East Anatolian Red is an important genetic resource of Turkey (Yardibi *et al*., 2009). Now-a-days, animal number of these breeds are rapidly decreasing and almost no purebred animals remained in farms because of wrong crossbred policies.

Anatolian Black and East Anatolian Red breeds have been conserved in Lalahan Livestock Central Institute and Eastern Anotolian Research Institute, respectively.

The objective of this study was to determine the genotypes and allelic frequency for kappa casein gene by use of PCR-RFLP technique and relationships between κ-casein (CSN3) genotypes and milk composition in some native cattle breeds of Turkey.

MATERIALS AND METHODS

Blood and milk sample collection and DNA extraction: A total of 50 bovine DNA samples were used in this study including the Anatolian Black (n = 20) and East Anatolian Red (30) native cattle breeds of Turkey.

Samples were collected from Middle and East Anatolia region of Turkey for Anatolian Black and East Anatolian Red, respectively. Blood samples (3 mL) were collected in sterile tubes containing EDTA anticoagulant from jaguler vein. Milk samples (15 mL) were collected from milking in sterile tubes. Genomic DNA was extracted from blood samples by using DNA exraction kit (Promega, USA).

Table 1: Fragment size for different genotypes after digestion (Soria *et al*., 2003)

Genotype	HindIII	HaeIII
AA	935	641+294
AB	935+520+415	641+294
AE	935	641+496+294+145
BB	520+415	641+294
BE	935+520+415	641+496+294+145
EE	935	496+294+145

Milk analysis: Milk samples were analyzed for protein, fat, lactose, solids, solids not fat content and density (kg m^{-3}) by using milk analyzer (Lactoscan Milk Analyzer 60, Bulgaria).

Polymerase chain reaction: A 935 bp fragment of bovine kappa casein gene exon 4 was amplified by Polymerase Chain Reaction (PCR) from genomic DNA using forward (5'-AGCGCTGTGAGAAAGATG-3') and reverse (5'-GTGCAACAACACTGGTAT-3') primers which were designed by Soria *et al*. (2003).

The PCR was performed in a 30 μL final volume containing 6 μL genomic DNA, 3 μL $MgCl_2$ (25 mM), 3 μL 10× buffer [(NH_4) $2SO_4$], 0.2 μL Taq DNA polimeraz (5 u μL^{-1}), 3 μL dNTP mix (2.5 mM), 1 μL forward primer (20 pM), 1 μL reverse primer (20 pM), 13 μL ddH_2O. Thermal cycling conditions were: 95°C for 5 min, 35 cycles of 95°C for 45 sec, 60°C for 45 sec, 72°C for 60 sec with final extension at 72°C for 5 min.

Restriction fragment length polymorphism: The PCR products were digested with the restriction enzymes HindIII and HaeIII to distinguish allelic variants A, B and E (Table 1 and Fig. 1).

Statistical analysis: Determinated genotypes for polymorphisms of animals were compared with milk protein, fat, lactose, solids, Solids Non Fat (SNF) contents and density by statistical analysis. Statistical analysis of milk composition in relation to CSN3 genotypes was carried using the GLM procedure (Minitab 15.1.1 by 2007). Differences between mean values of the traits were tested with Tukey multiple range test. The following linear model was applied to all the traits analysed:

$$Y_{ijklm} = \mu + a_i + b_j + c_k + d_l + e_{ijklm}$$

Where:

Y_{ijklm} = Observed value
μ = Trait mean
a_i = Genotype effect (i = 1, 2)
b_j = Lactation number effect (j = 1...4)

c_k = Calving age effect (k = 1...4)
d_l = Calving season effect (l = 1...4)
e_{ijklm} = Error

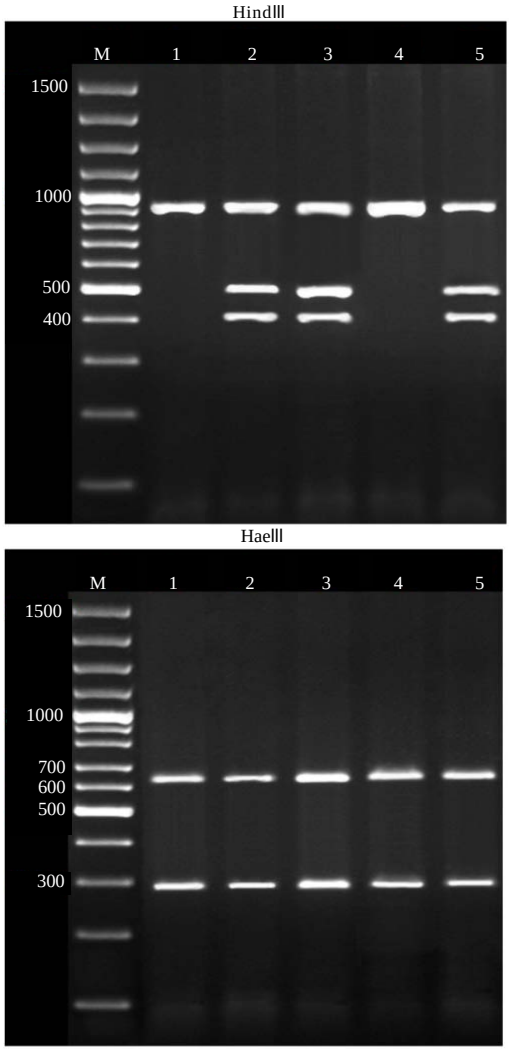

Fig. 1: Identification of CSN3 alleles by digestion with HindIII and HaeIII. Lane M: DNA molecular weight marker (100 bp); Lanes 1, 4 genotype AA; Lanes 2, 3, 5 genotype AB

RESULTS AND DISCUSSION

Genotype and allele frequencies distribution of Anatolian Black and East Anatolian Red breeds for CSN3 polymorphisms were shown in Table 2. Least square means and effects of genotypes on milk composition traits were shown in Table 3. In conclusion, only the genotypes AA and AB were observed in Anatolian Black and East Anatolian Red breeds. BB, AE, BE and EE gonotypes were not detected in these breeds. Genotype frequencies for *CSN3* gene were determined as AA = 0.500, AB = 0.500 for Anatolian Black and AA = 0.567, AB = 0.433 for East Anatolian Red.

Allele frequencies for *CSN3* gene were determined as A = 0.750, B = 0.250 for Anatolian Black and A = 0.783, B = 0.217 for East Anatolian Red (Table 2). CSN3 A allele frequency was found >B allele. CSN3 E allele which have been reported to be rare in most breeds (Erhardt, 1989) was not detected in Anatolian Black and East Anatolian Red breeds. According to the knowledge, this research is the first one reporting genotype and allele frequencies for *CSN3* gene in Anatolian Black and East Anatolian Red breeds.

The effects of genotypes on milk protein and solids non fat contents were found significant in East Anatolian Red ($p<0.05$) and also found close to significant in Anatolian Black ($p<0.1$). These results were similar to

Table 2: Genotype and allele frequencies

	Anatolian Black		East Anatolian Red	
Parameters	n	f	n	f
Genotype				
AA	10	0.500	17	0.567
AB	10	0.500	13	0.433
BB	-	-	-	-
AE	-	-	-	-
BE	-	-	-	-
Total	20	1.000	30	1.000
Allele				
A	30	0.750	47	0.783
B	10	0.250	13	0.217
E	-	-	-	-
Total	40	1.000	60	1.000

Table 3: Least square means of milk composition traits for different genotypes ($\bar{X} \pm S_{\bar{X}}$)

Traits	n	Fat (%)	SNF (%)	Density (kg m^{-3})	Protein (%)	Lactose (%)	Solids (%)
Anatolian Black							
AA	10	4.143±0.65	9.373±0.18	1033.30±0.81	3.421±0.07	5.148±0.10	0.7662±0.01
AB	10	5.017±0.55	9.784±0.15	1032.36±0.68	3.581±0.06	5.360±0.08	0.7955±0.01
p-value		0.308	0.092	0.379	0.073	0.117	0.125
East Anatolian Red							
AA	17	3.924±0.19	9.225±0.14^{b}	1032.86±0.32	3.368±0.05^{b}	5.058±0.08	0.7582±0.01
AB	13	4.241±0.21	9.672±0.16^{a}	1032.37±0.37	3.548±0.06^{a}	5.289±0.09	0.7900±0.01
p-value	-	0.280	0.044*	0.317	0.031*	0.057	0.061

*: $p<0.05$, ^{a,b}: Differences in same column are significant ($p<0.05$)

Ikonen *et al.* (1999b), Tsiaras *et al.* (2005) and Molina *et al.* (2006) who had reported that effects of CSN3 genotypes on protein content were significant and B allele responsible for higher protein content.

The effects of genotypes on lactose contents were also found close to significant in East Anatolian Red ($p<0.1$). The effects of genotypes on fat and solids contents and density were not found significant in Anatolian Black and East Anatolian Red. Present results agree with Tsiaras *et al.* (2005) who had reported effects of CSN3 genotypes on fat content and lactose content were not significant.

CONCLISION

Consequently, the effects of CSN3 polymorphisms on milk composition were found significant. The association between CSN3 genotypes and milk protein and SNF contents suggests that CSN3 polymorphism might be a useful marker for genotypic selection such as marker assisted selection. Selection of bulls carrying B allele could provide rapid genetic improvement for increasing milk quality.

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