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Genetic Variants of β-Lactoglobulin, α_{s1}-Casein and β-Casein of Milk in East Anatolian Red Cattle Breed

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β-Lactoglobulin (β-Lg), α_{s1}-casein (α_{s1}-Cn) and β-casein (β-Cn) polymorphisms of East Anatolian Red breed cattle raised in Turkey were studied by applying starch urea gel electrophoresis. In addition, milk yield, fat content and rennet clotting time were determined. Electrophoretic analysis showed that milk samples taken from 34 East Anatolian Red cattle breed had AA, AB and BB genotypes of β-Lg, BB and BC genotypes of α_{s1}-Cn and AA and AB genotypes of β-Cn. No differences were found for milk yield and fat content related with the genetic variants of β-Lg, α_{s1}-Cn and β-Cn ($p > 0.05$). There were significant differences for rennet clotting time of milk related with the genetic variants of β-Lg and β-Cn ($p < 0.05$) whereas no differences were found with the genetic variants of α_{s1}-Cn ($p > 0.05$). Therefore, it was concluded that selection of individual East Anatolian Red cows for AB genotype of β-Lg and AA genotype of β-Cn would be more appropriate, due to the fact that milk from these cows are associated with lower rennet clotting time.

Key Words: Bovine milk, Genetic polymorphism, Milk yield, Fat content, Rennet clotting time.

INTRODUCTION

Bovine milk proteins are divided into two groups, depending on their solubility at pH 4.6. The soluble fraction, named whey protein, is constituted by several different proteins; the most important ones are α-lactoglobulin (α-La) and β-lactoglobulin (β-Lg). The insoluble fraction is constituted of four different native caseins (Cn): α_{s1}-Cn, α_{s2}-Cn, β-Cn and κ-Cn[1]. The three caseins (α_{s1}-Cn, β-Cn and κ-Cn) and α-La and β-Lg have been found to be polymorphic and possible relationships between milk protein polymorphism and milk yield and milk composition have been widely studied[2-6]. However, the reports are not always in agreement and sometimes conflicting.

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The different genetic variants of milk proteins differ from each other by only a few amino acid substitutions or deletions within the polypeptide chain[7]. Consisting of seven genetic variants (A, B, C, D, E, F and G), β-Lg is the main component of whey proteins and is the first milk protein where genetic polymorphism was found in cows' milk[8]. Five genetic variants of α_{s1}-Cn, designated as A, D, B, C and E in order of decreasing electrophoretic mobility in alkaline gels containing urea and 2-mercaptoethanol, have been reported[8]. The recommended designations for the different variants of β-Cn are: A^1, A^2, A^3, B, C, D and E[7]. Aschaffenburg[9] discovered three variants, denoted as A, B and C. The A variant of β-Cn could be resolved into A^1, A^2 and A^3 under acidic conditions of gel electrophoresis[10].

The genetic variants of milk proteins have received great interest in dairy industry, in particular, for well-confirmed association with composition, rennet coagulation and cheese making properties of milk, which are of economic importance for cheese industry[11]. The BB variant of β-Lg is associated significantly with higher milk yield[12], higher fat[3,4,13,14], protein[3] and casein content[14]. Marziali and Ng-Kwai-Hang[15] and Ng-Kwai-Hang *et al.*[14] have found an association of β-Lg AA phenotype with significantly shorter rennet clotting time. However, Aaltonen and Antila[16] and Pagnacco and Caroli[17] could not find any relation between β-Lg polymorphism and fat contents and rennet clotting time of cows' milk. Genotypes of α_{s1}-Cn significantly influenced milk yield, fat yield and protein yield with the highest yields obtained for the genotype BB[3]. Ng-Kwai-Hang *et al.*[14] found the highest concentrations of fat, protein and casein in Holstein-Friesian cows' milk of phenotype A^1B for β-Cn. B variant of β-Cn is associated with shorter time of coagulating than the other variants[2,18].

Data about milk protein polymorphisms in East Anatolian Red breed were not available. Therefore, the purpose of this study was to determine β-lactoglobulin, α_{s1}-casein and β-casein polymorphisms of East Anatolian Red breed and to study the association of their genetic variants with milk yield, fat content and rennet clotting time.

EXPERIMENTAL

Individual milk samples (*ca.* 1000 mL) were collected from 34 East Anatolian Red cattle breed in the dairy herds of Erzurum farm of East Anatolian Agricultural Research Institution. The milk samples were taken on the 3rd and 4th month of calving during the morning milking. Cows in the same lactation number were chosen and fed on pasture completely. The samples were cooled and stored at 4 ºC until analyzed for β-Lg, α_{s1}-Cn and β-Cn genotypes and physicochemical parameters. All the analyses were replicated twice.

Starch urea gel electrophoresis at pH 8.6 was used for determination of genetic variants of the milk proteins according to Aschaffenburg and Michalak[19]. Before electrophoresis the milk samples were centrifuged at 2500 × g for 15 min to remove fat. The electrophoresis was achieved by using vertical slab gels that have the following dimensions: 120 mm long, 180 mm wide and 3 mm thick.

Lactation milk yield was determined by recording daily milk yield during the lactating period. The fat content of the milk samples were analyzed according to Kurt *et al.*[20]. The rennet clotting time of milk was determined according to IDF[21].

Statistical analysis: The predominant electrophoretic band of each allele was considered for phenotyping. The effect of phenotypic structure of milk proteins on chemical composition and rennet clotting time of the milk was screened with variance analysis of ANOVA. The statistical analyses were carried out by using MINITAB® software[22].

RESULTS AND DISCUSSION

Genetic polymorphism of β-lactoglobulin, α_{s1}-Cn and β-Cn: Electrophoretic analysis showed that milk samples taken from 34 East Anatolian Red cattle breed had AA, AB and BB genotypes of β-Lg. Semenenko *et al.*[23], Medvedeva[24] and Toome[25] have reported similar genotypes of β-Lg. Of the 34 East Anatolian Red cows, 20 showed BB genotypes (Table-1).

TABLE-1
VARIATIONS IN MILK YIELD AND FAT CONTENT WITH PROTEIN GENOTYPES

Genotypes	Number of cows	Milk yield (kg)	Fat content (%)
β-Lg			
AA	5	603.6 ± 89.3	4.74 ± 0.503
AB	9	666.1 ± 187.6	4.59 ± 0.298
BB	20	769.5 ± 293.0	4.77 ± 0.247
α_{s1}-Cn			
BB	18	726.7 ± 269.2	4.67 ± 0.293
BC	16	707.6 ± 238.3	4.77 ± 0.322
β-Cn			
AA	28	733.4 ± 268.6	4.71 ± 0.275
AB	6	644.7 ± 141.2	4.77 ± 0.465

East Anatolian Red breed had BB and BC genotypes of α_{s1}-Cn whereas AA, BB, CC, AB and BC genotypes of α_{s1}-Cn have been reported[26,27]. As can be seen in Table-1, of the 34 cows, 18 showed BB genotypes and 16 BC genotypes of α_{s1}-Cn.

Although AA, BB, CC, AB, AC and BC genotypes of β-Cn have been reported[28,29] only AA and AB genotypes were found in East Anatolian Red breed. 28 out of 34 cows had AA genotypes of β-Cn (Table-1).

Relationships between genotypes of β-Lg and milk yield or milk fat: Variations in milk yield or fat content according to genotypes of β-Lg are given in Table-1. The cows with BB genotype gave highest milk yield and fat content. But the differences between genotypes with respect to milk yield and fat content were not significant ($p > 0.05$). Similar results were reported by Üstdal *et al.*[30]. On the other hand, Matyukov[31] and Meyer *et al.*[11] found that genotypes of β-Lg had significant effects on milk yield and the cows with BB genotype gave the highest milk yield.

Unlike to present results, Buczynski *et al.*[32] found that the cows with AB genotype gave significantly higher fat content than the cows with AA genotype. However, Toome[25] and Samarineanu *et al.*[33] reported that AA genotype produced higher fat content.

Relationships between genotypes of α_{s1}-Cn and milk yield or milk fat: Variations in milk yield or fat content according to genotypes of α_{s1}-Cn are given in Table-1. Although the cows with BB genotype gave higher milk yield, it was observed that variations in genotypes of α_{s1}-Cn had no significant effects on milk yield or fat content ($p > 0.05$). Similarly no significant effects of genotypes of α_{s1}-Cn on milk yield were observed by Putz *et al.*[34] and Eenennaam and Medrano[5]. However, Ronda *et al.*[26], Marinchuk[35] and Lin *et al.*[36] reported that cows with BB genotype had higher milk yield than BC genotype. Similarly Ng-Kwai-Hang *et al.*[3] also reported higher milk yield with BB genotype. With respect to fat content, Macha and Mednanska[37] reported that the cows with BC genotype produced milk with higher fat content.

Relationships between genotypes of β-Cn and milk yield or milk fat: As can be seen in Table-1, AA genotype produced higher milk yield (733.4 kg) than AB genotype (644.7 kg). However, there was no significant differences between the two genotypes ($p > 0.05$). Additionally, variations in genotypes of β-Cn had no significant effect on fat content. Kamenskaya[28], Samarineanu *et al.*[29] and Özbeyaz *et al.*[38] reported that AA genotype produced higher milk yield.

Macha and Mednanska[37] found that the cows with AB genotype produced milk with higher fat content. On the other hand Bech and Kristiansen[39] reported that AA genotype produced higher fat content.

Relationships between genetic polymorphism of milk protins (β-lactoglobulin, α_{s1}-Cn and β-Cn) and the rennet clotting time (RCT) of milk: Mean values and standard deviations of RCT of East Anatolian Red milk were shown in Table-2. Significant differences were found for rennet clotting time of milk related with the genetic variants of β-Lg ($p < 0.05$). Lower rennet clotting time was obtained in the milk with AB phenotype of β-Lg than that milk with either AA or BB genotypes.

TABLE-2
VARIATIONS IN RENNET CLOTTING TIME (RCT) WITH PROTEIN GENOTYPES

Genotypes	Number of cows	RCT (min)
β-Lg		
AA	5	10.20 ± 7.33
AB	9	5.00 ± 1.32
BB	20	6.70 ± 2.43
α_{s1}-Cn		
BB	18	6.40 ± 4.24
BC	16	6.80 ± 3.46
β-Cn		
AA	28	5.50 ± 1.93
AB	6	10.16 ± 6.53

The reduction in rennet clotting time of milk results an increase in curd firmness with eliminating problems rising from the soft curd[40]. The effect of β-Lg polymorphism on rennet clotting time is controversial in literature. Marziali and Ng-Kwai-Hang[15] reported that significantly shorter rennet clotting time was obtained in Holstein cows' milk containing β-Lg AA genotype when compared with AB and BB phenotypes. However, Pagnacco and Caroli[17], Aaltonen and Antila[16], Lodes *et al.*[41], Ikonen *et al.*[42] and Hallén *et al.*[43] could not find significant differences between β-Lg phenotypes in terms of rennet clotting time of cows' milk.

No effect of the α_{s1}-Cn locus on rennet clotting time was found in the present study ($p > 0.05$). With respect to genetic variants of β-Cn significant differences were observed for rennet clotting time. Significantly shorter rennet clotting time was obtained in EAR cows' milk containing β-Cn AA genotype ($p < 0.05$). In agreement with our results Hallén *et al.*[42] found the β-Cn AA genotype to be associated with the good coagulating properties.

On the other hand, Graham *et al.*[18] and McLean[2] reported that B variant of β-Cn was associated with shorter rennet clotting time than the other variants. In contrast, in studies by Pagnacco and Caroli[17] and Ikonen *et al.*[42] the effect of β-Cn genotype was not significant for coagulation time.

Conclusion

It is well known that genetic polymorphism of milk proteins is associated with the composition and technological properties of the milk. Electorphoretic analysis showed that milk samples taken from 34 East Anatolian Redcattle breed had AA, AB and BB genotypes of β-Lg, BB and BC genotypes of α_{s1}-Cn and AA and AB genotypes of β-Cn. No differences were found for milk yield and fat content related with the genetic variants of β-Lg α_{s1}-Cn and β-Cn. There were significant differences for rennet clotting time of milk related with the genetic variants of β-Lg and β-Cn whereas no differences were found with the genetic variants of α_{s1}-Cn. Therefore, selection of individual EAR cows for AB genotype of β-Lg and AA genotype of β-Cn would be more appropriate, due to the fact that milk from these cows are associated with lower rennet clotting time.

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